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Pharmacotherapeutic Potential of leaf extracts of *Grewia multiflora* Juss.

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ABSTRACT

The maintenance of disease-free human existence on this planet has been made possible by traditional medicines, which are primarily made from plants. In this study, extracts of leaf of *Grewia multiflora* Juss. (Malvaceae) was pharmacotherapeutically investigated. The plant is said to be used against diabetes, liver diseases, bone fractures. Defatted residue of leaf powder was soxhlated to get chloroform, acetone, methanol and aqueous extracts in a common manner. A part of the residue obtained after chloroform extract was soxhlated with a solvent mixture methanol and water (70:30) to get the extract methanol(70). The content of methanol(70) was in excess in comparison to methanol and others. Extracts were subjected to phytochemical investigation, study for total flavonoid and phenolic contents. Methanol and methanol(70) extracts were investigated for hepatoprotective and antiantidiabetic activities. Suspension of the extract in 0.5% sodium carboxymethylcellulose was taken for investigation of hypoglycemic capability of extract on diabetic rodent by comparing with the glibenclamide. Me(70)[300mg/kg] was having excess hypoglycemic capability than Me[300mg/kg]. This activity corresponds to flavonoids and other phytochemicals in Me(70) & Me. Me(70) [300 mg/kg p.o.] due to its antioxidant properties decreased the enzyme elevation to normal in serum due to hepatic damage caused by carbon tetrachloride.

KEY TERMS: Pharmacotherapeutic, *Grewia multiflora* Juss., leaf extract, Methanol(70), antidiabetic, hepatoprotective

INTRODUCTION

Tribal and local people and vaidyas claimed the use of leaf juice of *Grewia multiflora* Juss towards treatment of diabetes particularly Type-2 diabetes mellitus and jaundice. The plant is called Bhansuli in Hindi and hadjodi in Odiya. *Grewia multiflora* Juss, a plant grown in the biosphere of Similipal forest territory was chosen for investigation for cure of high blood sugar and damage of liver using the Me(70) and Me extract of leaf. *Grewia multiflora* Juss. is a plant found spread throughout our country and other part of world.[1] For giving protection to liver, to increase appetite, diseases of heart, decrease high temperature, rheumatic fever, diarrhea and diabetes, leaf juice is favoured.[2] Leaves contain flavonoids, saponins, glycosides, terpenoids, steroids and phenols.[3] Leaf extracts of *Grewia multiflora* Juss. were studied pharmacologically. The plan was to study the antidiabetic and hepatoprotective potential of the plant particularly leaf.

MATERIAL AND METHOD:

Pharmaceutical high grade chemicals were purchased/ procured.

Plant material procurement:

Leaves of *Grewia multiflora* Juss were plucked freshly(5kg) from the Meru Matha area near Subarnarekha River in the biodiversity territory of Similipal forest, Dist-Mayurbhanj, Odisha in August 2018 (Figure1). We took the help of Botanical survey of India, Howrah, WB for authentication. A sample specimen was deposited in that herbarium.

Extraction:[4]

Leaves were dried in shade away from sun light. Dried leaves were grinded mechanically to get moderately coarse powder. The powder was subjected to soxhlation first by petroleum ether (60-80^{o}C) to defat the mass. The residue then successively extracted by chloroform (Cm). Residue remained after extraction with chloroform was divided in to two portions. One portion was subjected to successive extraction by acetone (Ac), methanol (Me) and purified water (Aq). The other portion was extracted with methanol (70)[Me(70)] (Figure 2). Methanol (70) was a mixture of two solvents containing methanol (70%) and purified water (30%). This was used considering its good solvent properties in extracting major active phytoconstituents.[5] The physical parameters of extracts are given in Table 1.

Test for presence of phytoconstituents:[6]

Following methods were adopted for analysis of secondary metabolites in the extracts. Methods included:

S N	Constituent	Test	S N	Constituent	Test
1-	Carbohydrate	:Molisch	6-	Flavonoids	:Alkaline
2-	Reducing Sugar	:Benedict's	7-	Alkaloids	:Dragendorff's
3-	Terpenoids	:Liebermann Burchard	8-	Steroids	:Liebermann Burchard
4	Tannins, phenols	:Ferric chloride	9-	Saponins	:Foam
5	Glycosides	:General	10-	Protein	:Ninhydrin

Flavonoid Content estimation (TFC):[7]

Method: Modified $ALCl_3$ spectroscopic method was followed. TFC of extract was counted in mg/100gm of quercetin.

Phenolic Content estimation(TPC):[8]

Method: TPC was accounted as mg/100gm of gallic acid when estimated by a method using Folin-Ciocalteu Reagent.

Quercetin and Gallic acid's estimation curves are shown in Figure 3 & Figure 4 respectively and the observations are noted in Table 2.

Acute Toxicity Study:[9]

Healthy Wistar albino rats of 200 gm weight(average) were maintained in standard approved conditions of light and darkness, temperature and humidity. Animals were acquainted to climatic and room conditions 4 days before test. CPCSEA necessities were fulfilled. The author had received the IAEC approval from SIPS, Jharpokharia, Mayurbhanj, Odisha, India (Regd.No.:-787/PO/ac/03/CPCSEA) for all experimental protocols, Vide approval No: A09/18/IAEC/SIPS. Acute toxicity study was conducted.

Twenty five (25) gram of Methanol(70) leaf extract was accurately weighed and 0.5% Na CMC solution was added, stirred, volume was made up to 100 ml for a suspension.

Method: OECD guidelines was followed for study. Suspensions of 0.4mL,08mL,1.6mL,2.4mL, 3.2mL, 4mL, 8mL and 12mL respectively [at doses of 100mg, 200mg, 400mg, 600mg, 800mg, 1000mg, 2000mg/kg and 3000mg/kg] were fed to separate groups of rats(n=6). 10 ml/kg b.w. (0.5 % W/V Na CMC) in distilled water was fed orally as control. The animals were observed for toxic reactions from 3 hrs till daily once for 7 days and extended to 20 days. The rats were examined for mortality, toxicity and behavior.

Selection of extracts for further study: Considering high yield, maximum content of phytochemicals, higher content of total flavonoids and phenolics, L-Me(70) and L-Me of leaf were taken for pharmacological investigations. 150mg/kg and 300mg/kg body weight per oral as the lower($1/20^{th}$) and higher($1/10^{th}$) therapeutic dose respectively were selected for further pharmacological studies.

Assessment of Antioxidant Activity (*in vitro*):[10]

Free radicals (DPPH, NO and $O2^-$) are scavenged by antioxidants. The antioxidant activity of selected leaf extract [L-Me(70) and L-Me] of *Grewia multiflora* Juss taken as 25, 50 and 100 µg/mL in methanol were studied by following three *in vitro* standard methods. 25, 50 and 100 µg/mL of ascorbic acid in methanol was used as standard.

Scavenging by DPPH:

Method: Methanolic solution of DPPH 0.1 mM was prepared by dissolving 4mg in 100mL methanol. DPPH solution(2mL) and test sample/ standard solution (2mL) were mixed and stored at RT for 30min in dark. Using a colorimeter at 517nm, the absorbance was taken and recorded as Mean % inhibition ±SD in Table 3.

Scavenging by Nitric oxide(NO):

Method: The reaction mixture (3mL) and the Extracts / Ascorbic acid solution were incubated at 25 ^{o}C for 150min. 1.5 mL above mixture and 1.5 mL Griss reagent were added. Using a colorimeter, at 546 nm the absorbance was taken and recorded as Mean % inhibition ±SD in Table 3.

Scavenging by Superoxide anion($O2^{-}$):

Method: The reagent mixture and 0.1mL of sample/standard solution were thoroughly mixed. 100 µM of phenazine methosulphate solution was added, incubated at 25 ^{o}C for 5min. Using a colorimeter, at 560 nm the absorbance was taken and recorded as Mean % inhibition ±SD in Table 3.

Screening for Antidiabetic Activity:

Me(70) and Me extracts were subjected to study of antidiabetic activities using Wistar albino rats. Standard methods using selected chemicals (Alloxan, Streptozotocin) for induction of diabetes were used.

General study procedure:

Wistar albino rats: 200gm(approximately) in six numbers per group were selected. Animals were maintained following approved laboratory methods. Animals were grouped. Extract and drug as suspension in vehicle were given as mg/kg b.w.

Gr –I : Control (solvent as 0.5 % Na CMC solution)
Gr -IA: alloxan control (150 i.p.) / Streptozotocin(60 i.p.)
Gr –II : Standard [Glibenclamide (0.07]
Gr –III: L-Me(150)
Gr –IV: L-Me(300)
Gr –V : L-Me(70)(150)
Gr –VI : L-Me(70)(300)

Diabetic study by Alloxan Model:[11]

Procedure: Animals (7groups × n=6) were kept without food but with water for 12 hours. Alloxan monohydrate was dissolved in ice cold normal saline. The solution (150 mg/kg) was injected intra-peritoneally to develop diabetes. However, the dose of alloxan was decided basing on individual weight of rat. Fasting BGL was determined 72 hours after alloxan injection. Rodents with hike in sugar level above 220mg/dL were considered for test. Gr -I rodents(nondiabetic) fed 1ml of control solution. Group-IA rodents were considered as positive control. Gr-II rodents were fed Glibenclamide (0.07/day), and Gr-III to VI rodents were fed the dose of extracts once per day orally for 12 days. Blood was collected as per standard procedure at the interval of days(0, 1, 3, 6, 9 & 12 days). Estimated sugar content in plasma was counted and noted in Table 4 and shown in Figure 5.

Diabetic study by Streptozotocin Model:[12]

Procedure: Animals (7groups × n=6) were kept without food but with water for 12 hours. Citrate buffer (0.01M, PH-4.5) was taken to dissolve STZ and ice cooled. The solution of STZ (60 mg/kg) was injected followed by oral intake of 5% glucose. After 3 days, Rodents with hike in sugar level above 250mg/dL were considered for

test. Gr-I rodents (nondiabetic) fed 1ml of control solution. Gr-IA rodents were considered as positive control. Gr-II rodents were fed glibenclamide (0.07), and Gr-III to VI rodents were fed the dose of extracts once per day orally for 12 days. Blood was collected as per standard procedure at the interval of days(0, 1, 3, 6, 9 & 12 days). Estimated sugar content in plasma was counted and noted in Table 5 and shown in Figure 6.

Body parameter estimation after 12th day:

Finding of B. W.: Initial B.W. and B.W. after 12th day were accounted by electronic balance and recorded in Table-6 and shown in Figure 7.

Biochemical Parameters:[13,14]

Blood parameters: Standard kits were considered for noting of value of Serum insulin, liver glycogen and Glycated haemoglobin which are recorded in Table 7.

Lipid content: Retro-orbital plexus of fainted rodent was made for drawing of blood by capillary, transferred to tubes with EDTA (3mg/mL). Separated plasma was analyzed for lipid fractions by Auto-Analyzer as noted in Table 8.

Liver parameter finding: Separated plasma was analyzed for liver related parameters by Auto-Analyzer as noted in Table 9.

Kidney parameter: Separated plasma was analyzed for kidney related parameters by Auto-Analyzer as noted in Table10.

Hepatoprotective Activity Study:[15,16]

Procedure: Adult male Wistar albino rats were grouped[7x(n=6)]. Gr-I considered as control(vehicle as liquid paraffin intraperitoneally). Gr-II to VII were given carbon tetrachloride in liquid paraffin (1:2) [1 ml/kg b.w. intraperitoneally] once in 3 days for 16 days to develop substantial damage to liver. Gr- II animals were left untreated but given only carbon tetrachloride as mentioned. Gr-III received the standard (Silymarin - 25 mg/kg, b.w., p.o) each day interval for 16 days. As it was observed in antidiabetic activity, the suspension of Me(70) and Me extract of leaf showed significant results at [300 mg/kg]. L-Me(70)[300] & L-Me[300] were ingested orally to the rodents of Gr-IV to V in alternate days for 16 days. Then rodents were sacrificed for collecting blood, serum was separated and ingested assayed for liver enzymes.

Estimation of liver content: Estimation was done using kits (Span Diagnostic Limited, Surat, India) for different liver content as noted in Table 11.

Histological Observation: A mixture was made containing picric acid, formaldehyde and 40% glacial acetic acid to wash liver tissues for fixation followed by dehydration using ethanol. Cleaned tissues (3-5 μm sections) taken on slide were firmly fixed in paraffin (M.P. 58-60° C) and stained[using haematoxylin-eosin]. The slide was observed under microscope and photos were taken (images in Figure 8) for analysis of liver condition during treatment. [17]

RESULT & DISCUSSION:

Phytoconstituents: The amount of L-Me(70) was found to be higher (17.35%) than L-Me (9.18%).Other extracts were having low yield. Most of the phytoconstituents were extracted in the mixed solvent [methanol(70)]. L-Me(70) showed position test for presence of glycosides, steroids, terpenoids, saponins, flavonoids, phenolics,

tannins and carotenoids whereas L-Me showed position test for presence of glycosides, saponins, flavonoids, steroids, terpenoids, phenolics.

TPC and TFC: Total Flavonoid & Phenolic content of L-Me(70) was higher than L-Me. Others contained less amount of flavonoids & phenolics.

Toxicity findings: As because there was no mortality up the dose of 3000 mg/kg B.W., the lethal dose of the extracts was quite high. The plant is safe to use orally. 150mg/kg and 300mg/kg B.W. *per oral* as the lower($1/20^{th}$) and higher($1/10^{th}$) therapeutic dose respectively were selected for further pharmacological studies.

Selection of extracts for further study: In case of leaf extracts, it was found that the yield % was higher in L-Me(70) than L-Me. Other extracts such as acetone and aqueous extract had very negligible yield. Similarly maximum phytochemicals were extracted in L-Me(70) followed by L-Me. Other two extracts contained less number of phytochemicals. Both L-Me(70) and Me(70) were rich in flavonoids and phenolic compounds. It was observed that the L-Me(70) was non toxic and safe to use in the quantity more than 3000mg/kg b.w. Thus it was decided to proceed further with L-Me(70) and L-Me of leaf for pharmacological investigation.

Antioxidant Assessment: In scavenging by DPPH, L-Me(70) showed 77.53% and Ascorbic acid showed 90.38% inhibition, in scavenging by NO, L-Me(70) showed 67.71% whereas ascorbic acid showed 74.07% and in scavenging by O2-, L-Me(70) showed 45.83% whereas ascorbic acid showed 58.04%. In each case the concentration of extract/standard was 100 µg/mL. L-Me showed less inhibition against all three free radicals.

Alloxan model findings towards diabetes study: On treatment with L-Me(70)[300], BGL was decreased gradually($p<0.01$) during treatment period [12 days]. L-Me[300] decreased($p< 0.05$) the BGL during treatment period [12 days]. The extracts[150] showed less results.

Streptozotocin model findings towards diabetes study: On treatment with L-ML(70)[300], BGL was decreased gradually($p<0.01$) during treatment period [12 days]. L-ML[300] decreased($p< 0.05$) the BGL during treatment period [12 days]. The extracts[150] showed less results.

Body parameters assessment:

B.W.: After induction of diabetes by Streptozotocin it was observed that the untreated positive control showed lower in B.W. No significant change was recognised in test and standard groups.

Biochemical parameters: Decrease of serum insulin and liver glycogen level and increase of GlyHb level during diabetes were restored by L-Me(70) and other extracts ($p<0.05$).

Lipid Profile: STZ control group showed increase in level of cholesterol, triglyceride and LDL and decrease in level of HDL. L-Me(70)[300] like standard caused restoration of triglyceride, LDL and HDL level ($p<0.01$) and also restoration of cholesterol level ($p<0.05$). L-Me[300] was effectively restored ($p<0.05$) HDL level. Other extracts showed non-significant results for restoration.

Liver parameter analysis: SGOT, SGPT and ALP content were increased in diabetes in rodents. L-Me(70)[300] like standard caused restoration of SGOT, SGPT

and ALP level (p<0.01). L-Me[300] also caused restoration (p<0.05). Other extracts showed non-significant results for restoration.

Kidney parameter analysis: Serum urea, creatinine and Uric acid content were increased in diabetes in rodents. L-Me(70)[300] like standard caused restoration of all parameters (p<0.01) except serum creatinine with low rate (p<0.05). L-Me[300] also caused restoration (p<0.05). Other extracts showed non-significant results for restoration.

Biochemical Parameters analysis during Hepatoprotective study: Liver damage in rodent caused increase in serum AST, ALT, ALP and total bilirubin levels(p<0.01) but decrease in protein level(p<0.01) as compared to normal rodents. Oral ingestion of L-Me(70)[300] restored the biochemical level(p<0.01). L-Me[300] showed significant (p<0.05) result against positive(CCl_4) control.

Results of histological observation: The slides showed normal hepatic cells with cytoplasm, nucleus, nucleolus and central vein in normal tissue (Figure 8a). Positive control(CCl_4) showed severe damage of cells in the liver (Figure 8b). Leaf extract-L-Me[300] showed partial restoration of damaged liver cells (Figure 8d). Standard drug Silymarin (25 mg/kg) and leaf extract-L-Me(70)[300] showed restoration of the altered histopathological changes (Figure 8c, Figure 8e) respectively.

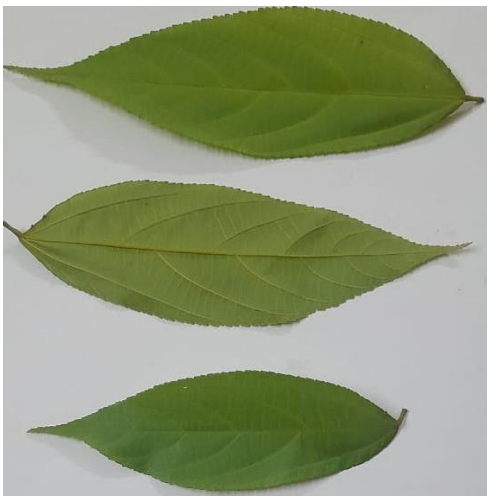

Fig.1. Leaves of selected plants.

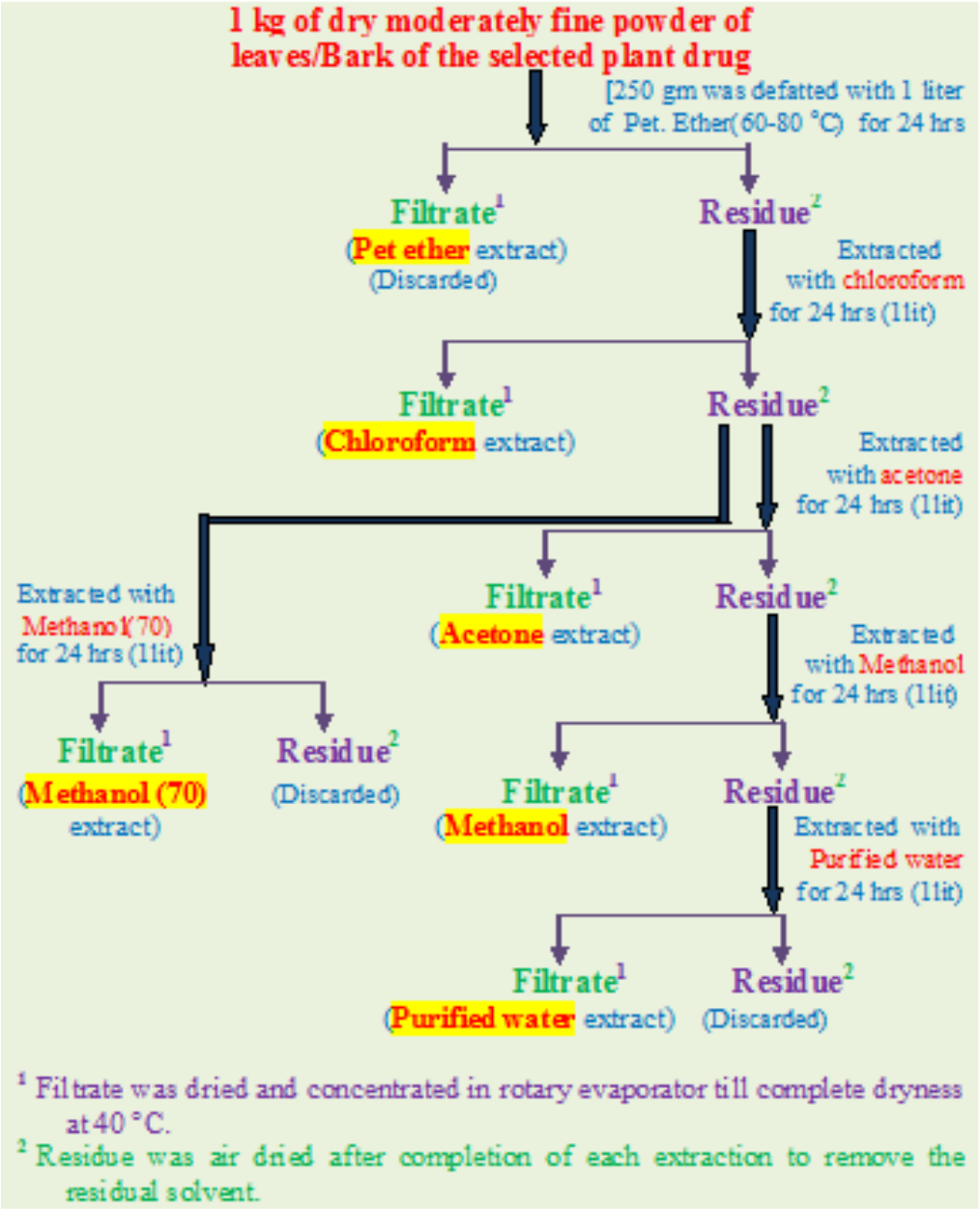

Fig.2. Details of steps of extraction process.

Table- 1: Physical parameters of leaf extracts:

Sl. No.	Type of extract	Code	Color	Consistency	% yield
1	Chloroform	L-Cm	Greenish black	Pillular	1.18 %
2	Acetone	L-Ac	Straw green	Pillular	2.03 %
3	Methanol	L-Me	Brownish green	Soft	9.18 %
4	Aqueous	L-Aq	Greenish black	Soft	7.62 %
5	Methanol(70)	L-Me(70)	Brownish green	Soft	17.35%

Table-2: TFC and TPC of leaf extracts:

Leaf Extract	TFC (mg/100gm) as QE	TPC (mg/100gm) as GAE
L-Cm	0.16±0.04	0.61±0.03
L-Ac	42.47±4.21	28.13±1.48
L-Me	98.56±3.75	108.47±3.12
L-Aq	02.26±1.14	01.14±0.42
L-Me(70)	102.42±3.18	115.48±3.72

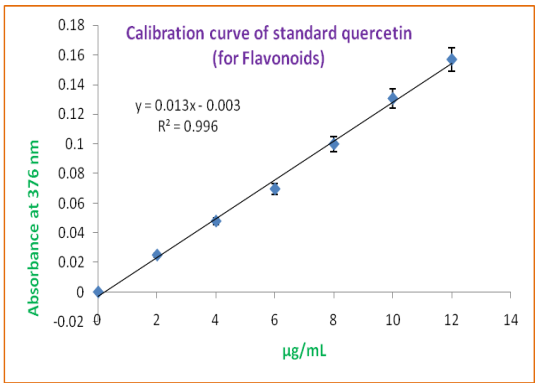

Fig.3. standard curve of Quercetin

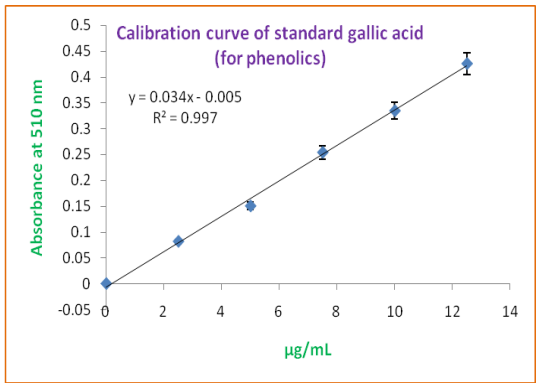

Fig.4. standard curve of Gallic acid

Table-3: Effect of selected leaf extracts on DPPH (Free radical), Nitric oxide (NO) and Superoxide Anion($O2^-$) scavenging:

Sample	Amount [µg/mL]	Mean % inhibition ±SD		
		DPPH (Free radical)	**Nitric oxide (NO)**	**Superoxide Anion($O2^-$)**
Control	Respective Reaction mixture	0.0	0.0	0.0
L-Me	25	16.8 ± 0.36	15.66 ± 0.45	9.82±0.56
	50	34.68 ± 0.13	27.99 ± 0.64	17.93±0.27
	100	58.47 ± 0.58	58.44 ± 0.9	29.06±0.63
L-Me(70)	25	21.24 ± 0.67	17.91 ± 0.94	17.56±0.18
	50	45.63 ± 1.09	32.4 ± 0.54	30.94±0.47
	100	77.53 ± 1.1	67.71±0.48	45.83±0.69
Ascorbic acid	25	71.6 ± 1.17	45.99 ± 0.56	47.01±0.87
	50	77.53 ± 1.1	56.52 ± 2.57	54.18±0.68
	100	90.38±0.87	74.07 ± 0.45	58.04±0.49

Table-4. Effect of leaf extracts on BGL in alloxan-induced diabetic rats:

Groups (n=6)	Treatment (mg/kg)	BGL (mg/dL) [Mean± SEM]					
		0day	1day	3day	6day	9day	12day
I	Control	92.65 ±2.67	93.74 ±2.86	93.15 ±3.14	93.28 ±2.87	93.52 ±3.24	92.42 ±3.18
IA	Alloxan control	219.47 ±2.68	234.23 ±3.52	225.14 ±4.17	204.27 ±5.67	191.17 ±4.01	185.72 ±5.78
II	Standard (0.07mg)	220.14 ±5.36	188.23 ±2.17	146.12 ±5.73*	121.75 ±4.35*	82.33 ±3.68^	72.53 ±4.51^
III	L-Me (150mg)	221.47 ±2.68	226.14 ±5.34	212.02 ±3.27	193.75 ±4.69	178.63 ±3.28	169.69 ±5.14
IV	L-Me (300mg)	222.14 ±4.39	210.26 ±3.58	192.01 ±5.13	144.74 ±4.27	116.28 ±6.35*	92.24 ±4.32*
V	L-Me(70) (150mg)	223.21 ±4.12	228.28 ±5.23	212.74 ±4.68	190.37 ±4.76	180.85 ±3.83	171.53 ±4.67
VI	L-Me(70) (300mg)	223.18 ±3.16	205.26 ±5.14	170.39 ±6.75	138.67 ±4.18*	85.36 ±5.76^	78.17 ±2.89^

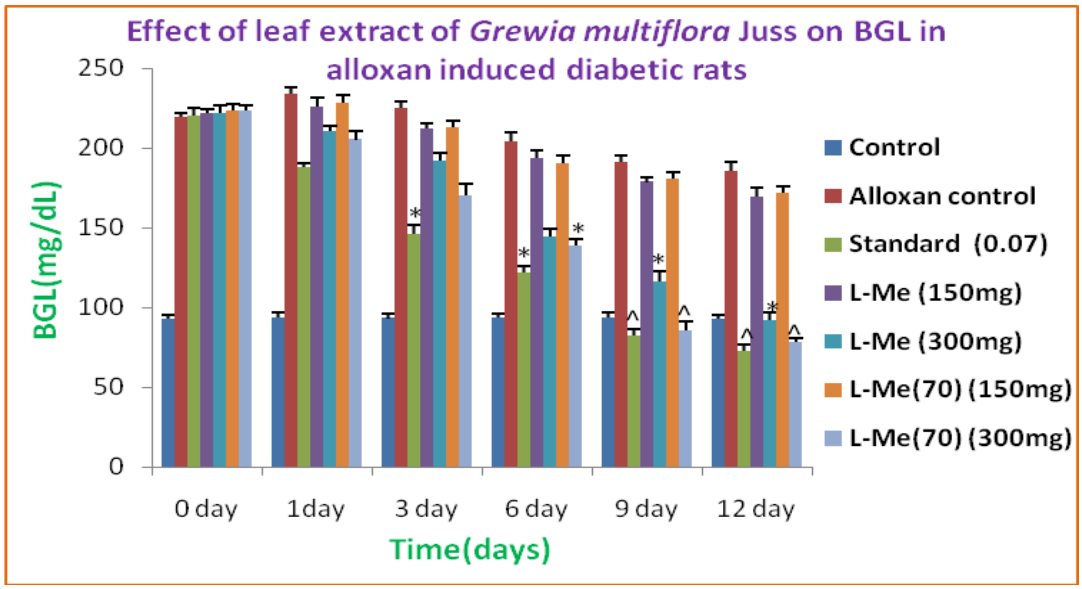

Fig.5. Histogram showing the effect of different leaf extracts on BGL in alloxan-induced diabetic rats.

Table-5: Effect of leaf extracts on BGL streptozotocin-induced diabetic rats:

Groups (n=6)	Treatment (mg/kg)	BGL (mg/dL) [Mean± SEM]					
		0day	1day	3day	6day	9day	12day
I	Control	92.32 ±4.23	94.75 ±3.78	95.45 ±5.28	95.65 ±4.07	94.87 ±3.24	93.17 ±4.17
IA	STZ control	268.75 ±3.62	262.23 ±4.10	240.41 ±5.18	223.63 ±4.57	205.18 ±3.68	196.07 ±2.16
II	Standard (0.07mg)	264.53 ±3.87	236.74 ±3.17	194.68 ±5.38	144.21 ±2.68*	82.29 ±4.21^	71.17 ±3.04^
III	L-Me (150mg)	266.68 ±3.75	259.43 ±4.12	234.15 ±5.38	207.86 ±3.86	192.72 ±4.51	176.14 ±3.70
IV	L-Me (300mg)	266.05 ±4.12	247.18 ±3.41	204.37 ±5.23	153.15 ±4.67	102.23 ±5.38*	87.67 ±3.22^
V	L-Me(70) (150mg)	267.21 ±3.52	256.34 ±5.03	232.75 ±4.34	204.63 ±3.38	189.28 ±2.11	174.19 ±5.89
VI	L-Me(70) (300mg)	269.52 ±3.69	244.41 ±4.12	205.74 ±6.24	153.32 ±4.78	99.76 ±4.59*	81.98 ±4.55^

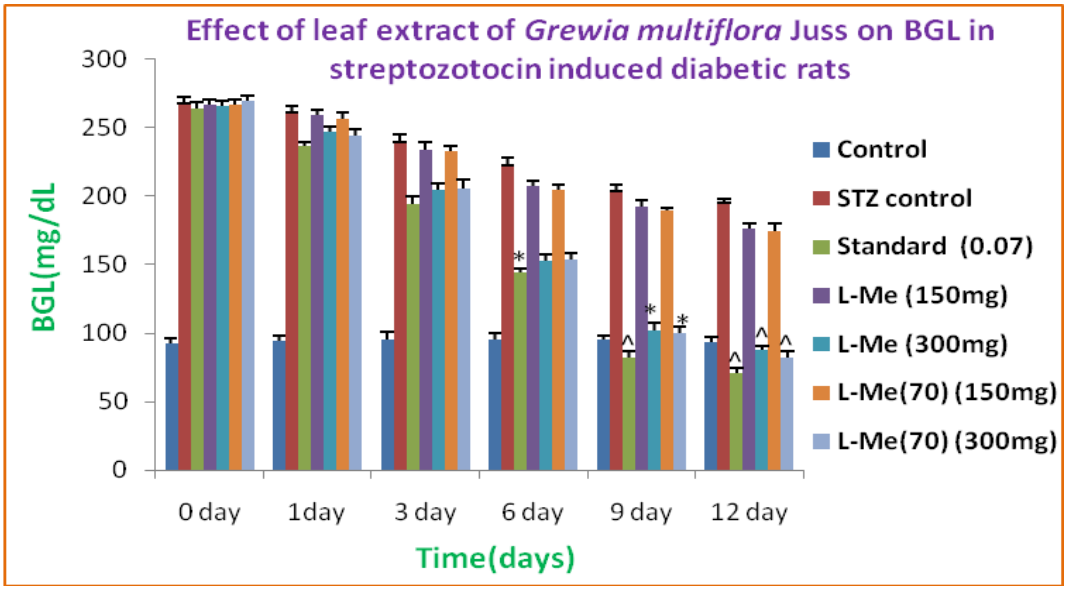

Fig.6. Histogram showing the effect of different leaf extracts on BGL on streptozotocin-induced diabetic rats.

Table-6: Effect of treatment with leaf extracts on change in body weight in diabetic rats:

Groups (n=6)	Treatment (mg/kg)	Body weight(gm) [Mean± SEM]	
		Day 0	Day 12
I	Control	209.36±8.15	210.03±4.28
IA	STZ control	203.74±6.38	185.42±4.65
II	Standard (0.07 mg)	198.37±6.15	201.25±7.03
III	L-Me (150mg)	202.78±8.23	195.13±6.45
IV	L-Me (300mg)	202.41±9.35	199.42±11.02
V	L-Me(70) (150mg)	202.35±10.65	197.14±8.46
VI	L-Me(70) (300mg)	201.36±9.21	195.52±8.39

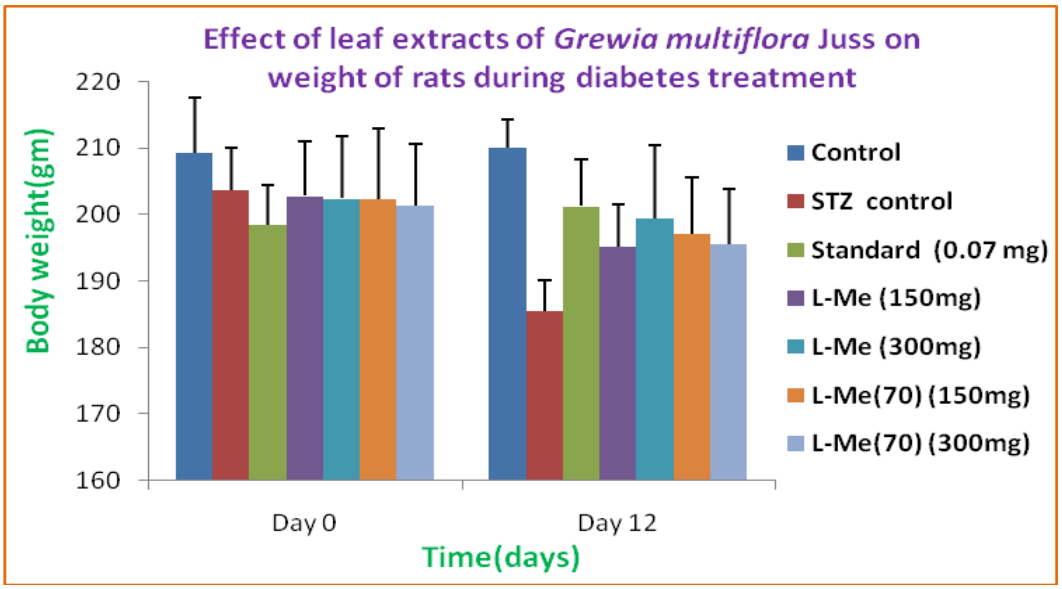

Fig.7. Histogram showing the effect of treatment by leaf extracts on change in body weight.

Table-7: Effect of treatment with leaf extracts on Haematological parameters in STZ-diabetes in rats:

Groups (n=6)	Treatment (mg/kg) (Mean± SEM)	Values are in (Mean± SEM)		
		Serum Insulin (µU/mL)	Gly Hb(%)	Liver Glycogen (mg/gm)
I	Control	14.02±0.87	3.94±0.24	8.84±0.32
IA	STZ control	6.85±0.69	10.36±0.13	5.24±0.74
II	Standard (0.07mg)	14.25±0.86$^{\wedge}$	3.83±0.62$^{\wedge}$	8.67±0.41$^{\wedge}$
III	L-Me (150mg)	10.53±0.98*	6.23±0.57*	6.87±0.21*
IV	L-Me (300mg)	11.28±0.74*	4.86±0.06*	7.22±0.14*
V	L-Me(70) (150mg)	10.96±0.82*	5.53±0.28*	6.42±0.32*
VI	L-Me(70) (300mg)	12.13±0.91*	4.37±0.13*	7.65±0.24*

Table-8: Effect of treatment with leaf extracts on lipid profiles in STZ-diabetes in rats:

Groups (n=6)	Treatment (mg/kg)	Values are in (Mean± SEM)			
		Cholesterol (mg/dL)	Triglycerides (mg/dL)	LDL (mg/dL)	HDL (mg/dL)
I	Control	110.36 ± 2.35	91.03 ± 2.04	66.14 ± 0.47	49.42 ± 0.29
IA	STZ control	223.56 ± 3.14	161.42 ± 1.06	139.14 ± 1.85	34.21 ± 0.65
II	Standard (0.07mg)	99.74 ± 1.42^	84.86 ± 0.57^	51.04 ± 0.25^	48.26 ± 0.74^
III	L-Me (150mg)	115.27 ± 2.03	96.54 ± 2.14	68.42 ± 0.65	40.43 ± 0.56
IV	L-Me (300mg)	109.25 ± 2.17	92.71 ± 1.05	59.31 ± 1.38	44.74 ± 0.43*
V	L-Me(70) (150mg)	113.52 ± 1.68	95.32 ± 0.68	66.74 ± 2.14	42.00 ± 1.13
VI	L-Me(70) (300mg)	107.78 ± 0.87*	89.16 ± 1.43^	56.35 ± 2.07^	46.13 ± 0.45^

Table-9: Effect of treatment with leaf extracts on biochemical parameter (Liver function tests) in STZ-diabetes in rats:

Groups (n=6)	Treatment (mg/kg)	Values are in (Mean± SEM)		
		SGOT (U/L)	SGPT (U/L)	ALP (U/L)
I	Control	32.24±2.03	23.72±0.38	87.36±3.35
IA	STZ control	70.74 ± 1.37	52.64 ± 2.12	180.48 ± 2.87
II	Standard (0.07 mg)	31.06± 1.28^	22.18± 1.07^	91.97 ± 1.48^
III	L-Me (150mg)	40.17 ± 0.87	30.41 ± 2.03	142.35 ± 1.52
IV	L-Me (300mg)	36.23 ± 0.46*	26.53 ± 0.69*	109.47 ± 0.87*
V	L-Me(70) (150mg)	39.78 ± 1.04	28.76 ±1.04	139.87 ± 1.68
VI	L-Me(70) (300mg)	34.06 ± 1.34^	24.06 ±0.87^	103.06 ± 2.03^

Table-10: Effect of treatment with leaf extracts on biochemical parameter (Kidney Function Test) in STZ-diabetes in rats:

Groups (n=6)	Treatment (mg/kg)	Values are in (Mean± SEM)		
		Serum urea (mg/dL)	Serum creatinine (mg/dL)	Uric acid (mg/dL)
I	Control	27.78±0.14	0.94±0.03	1.75± 0.26
IA	STZ control	77.25 ± 0.98	1.46 ± 0.42	4.89 ± 0.13

II	Standard (0.07 mg)	27.16 ± 0.42^	0.93 ± 0.27^	1.74 ± 0.12^
III	L-Me (150mg)	40.52 ± 0.67	1.19 ± 0.13	2.87 ± 0.14
IV	L-Me (300mg)	35.34 ± 1.15*	1.01 ± 0.04*	2.01 ± 0.75*
V	L-Me(70) (150mg)	37.73 ± 1.03	1.14 ± 0.17	2.23 ± 0.63
VI	L-Me(70) (300mg)	33.18 ± 0.57^	0.98 ± 0.07*	1.79 ± 0.61^

Table- 11: Effects of leaf extracts on hepatoprotective biochemical parameters:

	Values are in (Mean± SEM)(n=6)				
Parameters	Total bilirubin (mg/dL)	Total protein (g/dL)	ALP (IU/L)	AST (IU/L)	ALT (IU/L)
Control	0.32± 0.02	8.39± 0.53	76.21± 3.75	33.21± 2.37	49.32± 2.37
CCl_4 (1mL/kg)	0.75± 0.04#	5.81± 0.41#	323.93± 11.77#	125.01± 11.27#	230.59± 5.7#
Standard+ CCl_4	0.38± 0.03^	7.27± 0.33^	87.11± 10.83^	41.94± 2.99^	45.73± 5.83^
L-Me (300mg/kg) + CCl_4	0.51± 0.07*	6.23± 0.15*	152.16± 5.21*	73.76± 5.79*	88.36± 5.27*
L-Me(70) [300mg/kg]) + CCl_4	0.41± 0.06#	6.87± 0.59*	104.75± 7.58#	59.81± 3.85#	74.76± 2.19#

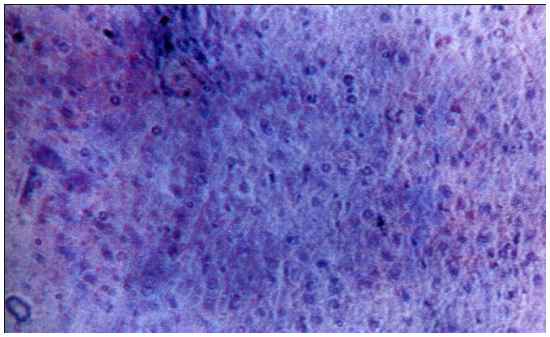

Fig.8a- Control

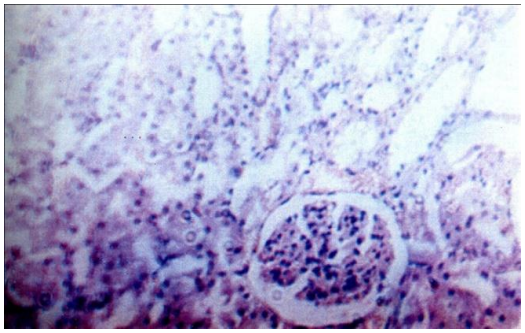

Fig.8b- CCl_4 induced

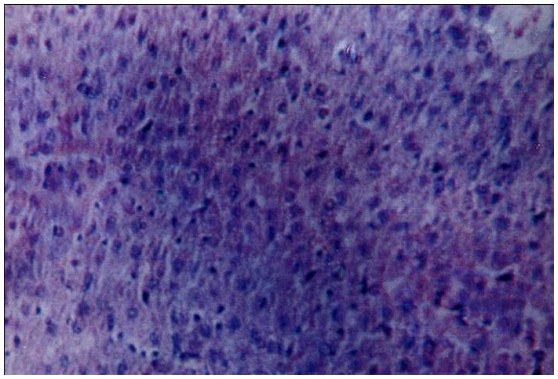

Fig.8c- Treatment with Standard [Silymarin (25 mg/kg)]

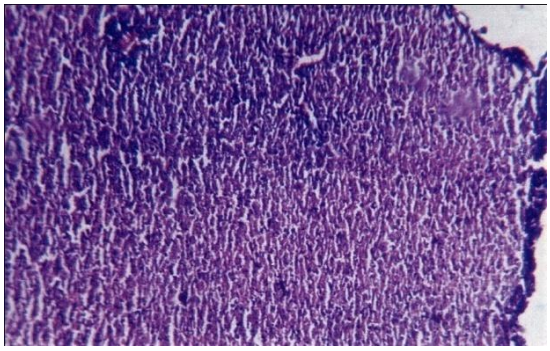

Fig.8d- Treatment with L-Me(300mg/kg)

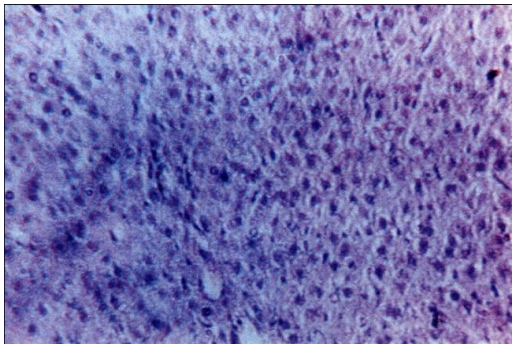

Fig.8e- Treatment with L-Me(70)[300mg/kg]

Fig. 8. Photomicroscope of liver sections from CCl_4 intoxicated rats after treatment with standard and extracts. (Fig.8a to Fig. 8e).

CONCLUSION:

Grewia multiflora Juss. (Malvaceae) is a well-known plant different parts (particularly leaves and stem bark) of which are used by local vaidyas and tribal people for treatment of many diseases such as jaundice, diabetes, bone fracture, microbial infections, wound etc. Lliterature findings showed very less study about this plant. The defatted leaf powder was extracted by soxhlation using Cm, Ac, Me, Aq. A portion of the residue after chloroform extraction was extracted with methanol and purified water mixture (70:30). Methanol(70) solvent was found to be more efficient towards extraction of major pharmaceutically active phytoconstituents. Methanol(70) extract and methanol extract were selected after thorough study of presence of phytochemicals, content of flavonoids and phenolics for pharmacological

potential study. Methanol(70) extract was found to have higher antioxidant potential than methanol extract. Diabetic animals which were administered with L-Me(70) extract of *Grewia multiflora* Juss at a dose of 300 mg/kg; normal blood glucose level was attended due to presence of living β-cells. These β-cells were influenced by *Grewia multiflora* Juss to show their insulin releasing capacity. Body weight in diabetes rodent decreased (p<0.05) due to an increase in muscle wasting in STZ model of diabetes. Bogy weight was unaltered when treated with L-Me(70) & L-Me extract of *Grewia multiflora* Juss at 300 mg/kg. Diabetes rats showed disturbances in serum parameters. L-Me(70) restored the normal values (p<0.05). STZ control group showed increase in level of cholesterol, triglyceride and LDL and decrease in level of HDL. SGOT, SGPT and ALP values and also Serum urea, creatinine and Uric acid content were increased in diabetes in rodents. L-Me(70)[300] like standard caused restoration of lipid constituents, liver parameters and kidney parameters These restorations were either(<0.01) or (<0.05). The present investigation revealed that L-Me(70) extract of *Grewia multiflora* Juss could be potential medicament for antidiabetic activity as its efficacy was evaluated in STZ induced type-2 diabetic rats and alloxan induced model. This is because of presence of higher concentration of flavonoids in L-Me(70) extract. Hepatic disorder caused by CCL_4 was effectively restored by Silymarin, where as L-Me(70)[300] was more potent in restoration (P<0.01) and also decrease of the elevated enzymes. Antioxidant property of the Methanol and methanol(70)extract might be responsible for hepatoprotective activity. Phytochemical analysis showed that L-Me(70) extract contained steroids, saponins, phenolics and flavonoids which showed higher antioxidant potential.

Statistical analysis:

All the data were presented as mean± SEM. ANOVA followed by Student *t*-test was adopted for evaluation of the statistical differences. Statistical significance on comparison with the control/positive control was considered by $^{\#}p$<0.001 highly significant, $^{\wedge}p$<0.01 more significant and $^{*}p$<0.05 significant.

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CONFLICT OF INTEREST: No conflict of interest

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ABBREVIATIONS:

QE	: Quercetin equivalent;	FCR	: Folin-ciocalteau's reagent;
GAE	: Gallic acid equivalents;	CMC	: Carboxymethylcellulose;
DPPH	: 2,2-diphenyl-1-picrylhydrazyl;	NO	: Nitric oxide;
O2-	: Superoxide anion;	BGL	: Blood glucose level;
STZ	: Streptozotocin;	TC	: Total cholesterol;
TG	:Triglyceride;	LDL	: Low-density lipoprotein;

HDL	: High-density lipoproteins;	i.p.	: Intraperitoneally
ALP	: Alkaline phosphatase;	B.W.	: Body weight
ALT	: Alanine transaminase;	AST	: Aspartate transaminase;
CCl4	: Carbon tetrachloride;	GlyHb	: Glycosylated hemoglobin;
Cm	: Chloroform;	Ac	: Acetone;
Me	: Methanol;	Aq	:Aqueous;
TFC	: Total flavonoid content;	TPC	: Total phenolic content;

SGPT :Serum glutamic pyruvic transaminase; p.o. : Per oral

SGOT: Serum glutamic-oxaloacetic transaminase;

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