



ANTIDIABETIC ACTIVITY OF METHANOLIC LEAF EXTRACT OF *DIOSPYROS FERREA* (WILLD) BAKH. IN STREPTOZOTOCIN INDUCED DIABETIC RATS

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ABSTRACT

The present study was designated to evaluate the antidiabetic activity of *Diospyros ferrea* methanolic leaf extract in Streptozotocin induced diabetic rats for 21 days. The leaf extract at high dose (400 mg/kg) exhibited significant anti hyperglycemic activity than leaf extract at low dose (200 mg/kg) in diabetic rats. The leaf extracts showed beneficial effect on parameters like blood glucose, serum lipid profile, serum protein, serum urea, uric acid, creatinine, Glycosylated hemoglobin, liver glycogen, Liver antioxidant enzymes and Serum AST and ALT levels.

KEYWORDS : *Diospyros ferrea* leaf, Anti diabetic activity, Albino rats, Streptozotocin, Blood parameters,

Introduction

The genus *Diospyros* species [Ebenaceae] were growing in subtropical and tropical areas of the China, India, Indonesia and the Malay Peninsula, consists of ca 240 sps, 59 of which are distributed in India [1]. Many species of *Diospyros* are medicinal and known to accumulate substantial amounts of naphthoquinones [2,3]. *Diospyros* species has been reported to have pharmacological applications arising from its extensive folkloric uses. The plant *Diospyros ferrea* is a bushy shrub widely distributed in Orissa and Southern India [4]. It is used to treat infertility and also shows anticancer, antimicrobial, diuretic activities along with germicidal activity [5,6,7]. Diabetes mellitus is a clinical syndrome characterized by hyperglycemia caused by insulin deficiency or by a resistance to the action of insulin at the cellular level. In modern medicine no satisfactory effective therapy is still available to cure diabetes mellitus. There is an increasing demand by patients to use natural products with antidiabetic activity due to side effects associated with the use of insulin and oral hypoglycemic agents [8,9,10].

Materials and Methods

Plant Material

The basic plant material of *Diospyros ferrea* (Wild) Bakh. leaf was collected from hill slopes of Tirumala, Andhra Pradesh, India and authenticated by Dr. A. Ravi kiran, BSI, Coimbatore.

Methanolic Extraction

The leaves were collected and shade dried and were subjected to pulverization to get coarse powder. The coarsely powder leaf (1 kg) of *Diospyros ferrea* was used for extraction with methanol in Soxhlet apparatus. The extract was evaporated to dryness under vacuum and dried in vacuum desiccator (15.5% w/w).

Animals

Male albino rats of Wistar strain weighing about 150-250 g were used in the study. The animals were housed in group of 6 rats per cage and maintained under standard laboratory conditions at 24±2°C in light controlled room [12 hrs dark and 12 hrs night] and provided commercial pellet diet.

Induction of Diabetes

In the present study, diabetes was induced by single intravenous injection of Streptozotocin (60mg/Kg) [11]. The rats were allowed to drink 5 % glucose solution overnight to overcome the drug induced hypoglycemia. Streptozotocin was first weighed individually for each animal according to the body weight and then solubilized with 0.2ml saline (154mM NaCl) just prior to injection. Treatment with plant extracts was started 48 h after Streptozotocin injection.

Acute toxicity studies

Acute oral toxicity study was performed as per organization for

Economic Cooperation and Development [OECD] guidelines 423. Acute toxicity was determined according to the method of Litchfield and Wilcoxon [12]. Stepwise dose of methanolic extract of *Diospyros ferrea* leaf [50 mg/Kg-2000 mg/Kg b.wt.], was administered. Animals were observed individually during the first 30 minutes and periodically during the first 24 hrs, with special attention given during the first 4 hours and daily thereafter, for total of 14 days. The dose 2000 mg/Kg was found to be safe and no toxicity was observed. There were no toxic effects of mortality observed up to 14 days. The LD50 cut off value found to be 2000 mg/Kg. For evaluation of anti-diabetic activity two dose levels were selected i.e., first dose is one-tenth of LD50 cut off value and second dose was twice that off one-tenth dose [200 mg/Kg and 400 mg/Kg. p.o.single dose].

Experimental Design. Five groups of rats, six in each received the following treatment schedule shown in table-1

Table-1-Experimental design with treatment schedule

Group	Treatment schedule
Group-I	Normal, untreated rats
Group-II	Diabetic Control rats[60mg/Kg of body weight]
Group-III	Diabetic Control rats given Glibenclamide[0.5mg/Kg of body weight]
Group-IV	Diabetic Control rats given MEDFL [200mg/Kg body weight]
Group-V	Diabetic Control rats given MEDFL [400mg/Kg body weight]

Collection of Blood Sample and Estimation of blood parameters

Blood samples were drawn from tail tip of rat at weekly intervals till the end of study (21 days) for blood glucose. Blood glucose was estimated by glucose oxidase peroxidase method [13]. On day 21st, blood was collected from retro-orbital plexus under mild ether anesthesia using a glass capillary tube. Serum was separated and analyzed for serum cholesterol [14], serum triglycerides by enzymatic DHBS colorimetric method [Fossati and Prencipe [15], Serum urea [16], Serum uric acid [17], Serum creatinine [18] and Jaffe [19]. Serum AST and ALT levels also estimated through Reitman and Frankel [20] method. Liver glycogen was estimated by using anthrone reagent [21,22]. Liver endogenous antioxidant enzymes such as Catalase [23] reduced Glutathione [24] and Lipid peroxidation [25] were estimated.

Statistical Analysis: All the values are expressed as Mean ± S.E.M. The data was analyzed for ANOVA and post hoc Dennett's t-test. The results were considered statistically significant when <0.05. The statistical analysis was carried out using Graph pad instat 3.0 software.

Results:

Effect of methanolic extract of *Diospyros ferrea* leaf on serum glucose levels in normal animal is shown in the Table-2 and Fig-1. In the animals treated with Streptozotocin [G-II] [150 mg/Kg, i.p, single dose] a significant increase in the serum glucose levels were observed on the 0th, 7th, 14th and 21st day, when compared to the normal animals [G-I]. This indicates that Streptozotocin induced group is having persistent diabetes mellitus.

Group-III, that received standard drug [Glibenclamide, 0.5 mg/Kg, p.o, once daily], there was significant decrease in the serum glucose levels on the 0th, 7th, 14th and 21st day, when compared to the diabetic control group. In standard group, the serum glucose levels approximately reached the normal on 21st day.

On administration of MEDFL [1 and 2 gm/Kg, p.o, once daily] there was a significant decrease in the serum glucose levels on 0th, 7th, 14th and 21st day, when compared to diabetic control group [G-II]. In both the groups [IV and V], the glucose levels were approximately equal to normal blood glucose levels on 21st day. These results suggest the antidiabetic activity of *Diospyros ferrea* leaf.

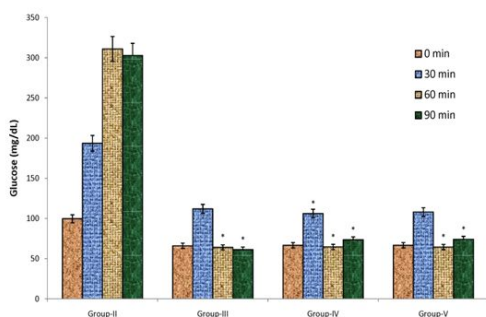


Fig-1: Effect of MEDFL on Serum Glucose

Table-2: Effect of MEDFL on Serum Glucose

Group	Treatment	Serum Glucose mg/dL			
		0 day	7 th day	14 th Day	21 st day
GROUP-I	Normal	90.16 ± 1.579	90.5 ± 1.668	90.83 ± 1.302	88.5 ± 0.763
GROUP-II	Diabetic Control [60 mg/Kg. i. p]	309.16 ± 2.750 ⁺⁺⁺	319.33 ± 0.802 ⁺⁺⁺	330.16 ± 3.763 ⁺⁺⁺	320.66 ± 0.881 ⁺⁺⁺
GROUP-III	Glibenclamide [0.5 mg/Kg p. o.]	307.83 ± 3.229	232.5 ± 1.455 ^{***}	165.33 ± 1.667 ^{***}	114.66 ± 2.186 ^{***}
GROUP-IV	MEDFL[200 mg/Kg p. o.]	310.16 ± 3.420	225.16 ± 2.182 ^{***}	174.83 ± 1.887 ^{***}	144.83 ± 1.641 ^{***}
GROUP-V	MEDFL [400 mg/Kg p.o.]	308.16 ± 4.468	244.5 ± 1.875 ^{***}	173.16 ± 1.195 ^{***}	127.83 ± 1.778 ^{***}

All values were expressed as Mean ± SEM. n=6

+++ means p<0.001, ++ means p<0.01, + means p<0.05 when compared with Normal Control.

*** means p<0.001, ** means p<0.01, * means p<0.05 when compared with diabetic control

Effect of methanolic extract of *Diospyros ferrea* leaf on cholesterol levels in Streptozotocin induced diabetic rats shown in the Table-3 and Fig-2. A significant increase in serum cholesterol level was observed in rats treated with Streptozotocin [G-II] on 21st day, when compared to normal group [G-I]. The group-III rats treated with standard drug [Glibenclamide, 0.5 mg/Kg, p.o, once daily] showed a significant decrease in serum cholesterol levels on 21st day, when compared to the diabetic control group [G-II]. The groups [IV and V] receiving MEDFL at a dose of 200 and 400 mg/Kg showed a significant decrease in serum cholesterol levels, when compared to diabetic control group [G-II]. The serum cholesterol levels were

decreased on 21st day in groups III, IV, and V. These values suggest that MEDFL had cholesterol lowering activity.

Effect of methanolic extract of *Diospyros ferrea* leaf on triglycerides levels in Streptozotocin induced diabetic rats shown in the Table-3. A significant increase in serum triglyceride level was observed in rats treated with Streptozotocin [G-II] on 21st day, when compared to normal group [G-I]. The group-III rats treated with standard drug [Glibenclamide, 0.5 mg/Kg, p.o, once daily] showed a significant decrease in serum triglyceride levels on 21st day, when compared to the diabetic control group [G-II]. The groups [IV and V] receiving MEDFL at a dose of 200 and 400 mg/Kg showed a significant decrease in serum triglyceride levels, when compared to diabetic control group [G-II]. The serum triglyceride levels were decreased on 21st day in groups III, IV, and V. These values suggest that MEDFL had triglyceride lowering activity.

Effect of methanolic extract of *Diospyros ferrea* leaf on HDL levels in Streptozotocin induced diabetic rats shown in the Table-3. A significant increase in serum HDL level was observed in rats treated with Streptozotocin [G-II] on 21st day, when compared to normal group [G-I]. The group-III rats treated with standard drug [Glibenclamide, 0.5 mg/Kg, p.o, once daily] showed a significant decrease in serum HDL levels on 21st day, when compared to the diabetic control group [G-II]. The groups [IV and V] receiving MEDFL at a dose of 200 and 400 mg/Kg showed a significant decrease in serum HDL levels, when compared to diabetic control group [G-II]. The serum HDL levels were decreased on 21st day in groups III, IV, and V. These values suggest that MEDFL had HDL lowering activity.

Effect of methanolic extract of *Diospyros ferrea* leaf on LDL levels in Streptozotocin induced diabetic rats shown in the Table-3. A significant increase in serum LDL level was observed in rats treated with Streptozotocin [G-II] on 21st day, when compared to normal group [G-I]. The group-III rats treated with standard drug [Glibenclamide, 0.5 mg/Kg, p.o, once daily] showed a significant decrease in serum LDL levels on 21st day, when compared to the diabetic control group [G-II]. The groups [IV and V] receiving MEDFL at a dose of 200 and 400 mg/Kg showed a significant decrease in serum LDL levels, when compared to diabetic control group [G-II]. The serum LDL levels were decreased on 21st day in groups III, IV, and V. These values suggest that MEDFL had LDL lowering activity.

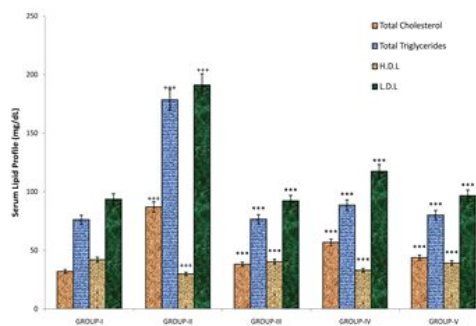


Fig:2 Effect of MEDFL on Serum Cholesterol, Triglycerides, HDL and LDL

Table-3: Effect of MEDFL on Serum Cholesterol, Triglycerides, HDL and LDL Cholesterol

All values were expressed as Mean ± SEM. n=6

Group	Treatment	Serum Lipid Profile			
		Total Cholesterol mg/dl	Total Triglycerides mg/dl	H.D.L mg/dl	L.D.L mg/dl
GROUP-I	Normal	32.0 ± 0.894	76.16 ± 1.014	42.0 ± 0.577	93.66 ± 1.430
GROUP-II	Diabetic Control [60 mg/Kg. i. p]	87.0 ± 0.894 ⁺⁺⁺	178.5 ± 0.846 ⁺⁺⁺	29.83 ± 0.477 ⁺⁺⁺	191.16 ± 1.815 ⁺⁺⁺

GROUP-III	Glibenclamide [0.5 mg/Kg p. o.]	38.16±1.014 ^{***}	76.66±0.881 ^{***}	40.33±0.494 ^{***}	92.33±1.333 ^{***}
GROUP-IV	MEDFL[200 mg/Kg p. o.]	56.83±2.056 ^{***}	88.66±1.202 ^{***}	33.0±0.577 ^{***}	117.5±0.763 ^{***}
GROUP-V	MEDFL [400 mg/Kg p.o.]	43.83±0.945 ^{***}	80.16±0.477 ^{***}	39.16±0.477 ^{***}	96.66±1.256 ^{***}

All values were expressed as Mean ± SEM. n=6

*** means p<0.001, ** means p<0.01, + means p<0.05 when compared with Normal Control.
 *** means p<0.001, ** means p<0.01, + means p<0.05 when compared with diabetic control.

Effect of methanolic extract of *Diospyros ferrea* leaf on urea levels in Streptozotocin induced diabetic rats shown in the Table-4 and Fig-3. Significant increase in serum urea was observed in rats treated with Streptozotocin [G-II] on 21st day when compared to normal group [G-I]. The group-III rats treated with standard drug [Glibenclamide, 0.5 mg/Kg.p.o once daily] showed a significant decrease in serum urea levels on on21st day when compared to the diabetic control group[G-II]. The groups [IV and V] receiving MEDFL at a dose of 200 and 400 mg/Kg showed a significant decrease in serum urea levels, when compared to diabetic control group [G-II]. The serum urea levels were decreased on 21st day in groups III, IV and V.

Effect of methanolic extract of *Diospyros ferrea* leaf on uric acid levels in streptozotocin induced diabetic rats shown in the Table-4 and Fig-3. A significant increase in serum uric acid was observed in rats treated with streptozotocin [G-II] on 21st day when compared to normal group [G-I]. The group-III rats treated with standard drug [glibenclamide, 0.5 mg/Kg.p.o once daily] showed a significant decrease in serum uric acid levels on 21st day when compared to the diabetic control group[G-II]. The groups [IV and V] receiving MEDFL at a dose of 200 and 400 mg/Kg showed a significant decrease in serum uric acid levels, when compared to diabetic control group [G-II]. The serum uric acid levels were decreased on 21st day in groups III, IV and V.

Effect of methanolic extract of *Diospyros ferrea* leaf on creatinine levels in streptozotocin induced diabetic rats shown in the Table-4 and Fig-3. A significant increase in serum creatinine was observed in rats treated with streptozotocin [G-II] on 21st day when compared to normal group [G-I]. The group-III rats treated with standard drug [Glibenclamide, 0.5 mg/Kg.p.o once daily] showed a significant decrease in serum creatinine levels on 21st day when compared to the diabetic control group[G-II]. The groups [IV and V] receiving MEDFL at a dose of 200 and 400 mg/Kg showed a significant decrease in serum creatinine levels, when compared to diabetic control group [G-II]. The serum creatinine levels were decreased on 21st day in groups III, IV and V.

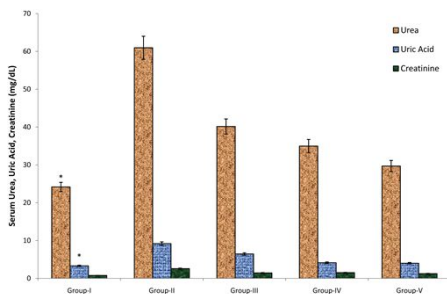


Fig.3. Effect of MEDFL on Serum Urea, Uric acid and Creatinine

Table-4: Effect of MEDFL on Serum Urea, Uric acid and Creatinine

Group	Treatment	Urea	Uric Acid	Creatinine
Group-I	Normal	24.178±1.905 *	3.326±0.352 *	0.785±0.087

Group-II	Diabetic Control [60mg/Kg. i. p]	60.955±1.510	9.196±1.222	2.600±0.120
Group-III	Glibenclamide[0.5mg/Kg p. o.]	40.145±1.768	6.451±0.509	1.456±0.100
Group-IV	MEDFL [200mg/Kg p. o.]	34.980±2.057	4.146±0.932	1.526±0.031
Group-V	MEDFL [400mg/Kg p. o.]	29.696±1.406	4.020±0.015	1.263±0.036

All values were expressed as Mean ± SEM. n=6

*** means p<0.001, ** means p<0.01, + means p<0.05 when compared with Normal Control
 *** means p<0.001, ** means p<0.01, + means p<0.05 when compared with diabetic control

Effect of Methanolic extract of *Diospyros ferrea* leaf on AST levels in Streptozotocin induced diabetic rats shown in the Table-5 and Fig-4. Rats treated with Streptozotocin [G-II] showed a significant increase in AST levels on 21st day, when compared to normal group [G-I]. The group [III] rats treated with standard drug showed a significant decrease in AST levels on 21st day, when compared to diabetic control [G-II]. The groups [IV and V] receiving MEDFL [200 and 400 mg/Kg] showed a significant decrease in AST levels on 21st day, when compared to diabetic control group [G-II].

Effect of methanolic extract of *Diospyros ferrea* leaf on ALT levels in Streptozotocin induced diabetic rats shown in the Table-5 and Fig-4. Rats treated with Streptozotocin [G-II] showed a significant increase in ALT levels on 21st day, when compared to normal group [G-I]. The group-III rats treated with standard [Glibenclamide 0.5 mg/Kg. p.o] drug showed a significant decrease in ALT levels when compared to diabetic control [G-II]. The group [IV and V] rats treated with MEDFL [200 and 400 mg/Kg] showed a significant decrease in ALT levels on on 21st day, when compared to diabetic control group [G-II]. In the group treated with high dose [400 mg/Kg] the ALT level was almost normal on 21st day.

Effect of methanolic extract of *Diospyros ferrea* leaf on total protein levels in Streptozotocin induced diabetic rats shown in the Table-5 and Fig-4. A significant decrease in serum total protein was observed in rats treated with Streptozotocin [G-II] on 21st day when compared to normal group [G-I]. The group-III rats treated with standard drug [Glibenclamide, 0.5 mg/Kg.p.o once daily] showed a significant increase in serum total protein levels on 21st day when compared to the diabetic control group [G-II]. The groups [IV and V] receiving MEDFL at a dose of 200 and 400 mg/Kg showed a significant increase in serum total protein levels, when compared to diabetic control group [G-II]. The serum total protein levels were increased on 21st day in groups III, IV and V.

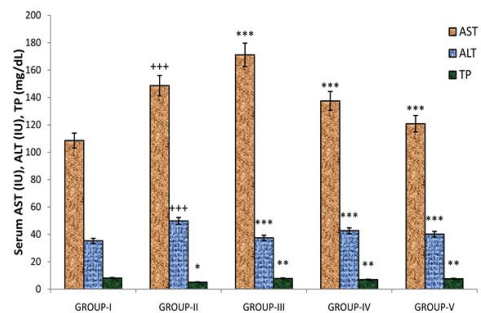


Fig 4: Effect of MEDFL on Serum AST, ALT and Total Protein level

Table-5: Effect of MEDFL on Serum AST, ALT and Total Protein level

Group	Treatment	Serum AST, ALT and Proteins		
		AST[IU]	ALT[IU]	TP[mg/dl]
GROUP-I	Normal	108.5 ± 1.176	35.33 ± 0.802	8.12 ± 0.063
GROUP-II	Diabetic Control [60 mg/Kg. i. p]	148.66 ± 1.054 ^{***}	49.83 ± 0.600 ^{***}	5.08 ± 0.171 ⁺

GROUP-III	Glibenclamide [0.5 mg/Kg p. o.]	171.16 ± 0.945 ^{***}	37.5 ± 0.562 ^{***}	7.82 ± 0.321 ^{**}
GROUP-IV	MEDFL[200mg/Kg p.o.]	137.5 ± 0.763 ^{***}	42.66 ± 1.282 ^{***}	6.98 ± 0.142 ^{**}
GROUP-V	MEDFL [400 mg/Kg p.o.]	120.83 ± 1.167 ^{***}	40.16 ± 0.703 ^{***}	7.62 ± 0.014 ^{**}

All values were expressed as Mean ± SEM. n=6
^{***} means p<0.001, ^{**} means p<0.01, ⁺ means p<0.05 when compared with Normal Control.
^{***} means p<0.001, ^{**} means p<0.01, ⁺ means p<0.05 ^{ns} means not significant when compared with diabetic control

Effect of Methanolic extract of *Diospyros ferrea* leaf on Glycosylated haemoglobins levels in Streptozotocin induced diabetic rats shown in the Table-6. In group treated with Streptozotocin [G-II] [60 mg/Kg, i.p, single dose] a significant increase in Glycosylated haemoglobin levels on 21st day was observed when compared to the normal animals [G-I]. In group-III, that received standard drug [Glibenclamide, 0.5 mg/Kg, p.o., once daily] there was significant decrease in the Glycosylated haemoglobin levels, when compared to the diabetic control group [G-II]. On administration of MEDFL [200 and 400 mg/Kg, p.o., once daily] to group IV and V, there was a significant decrease in the glycosylated haemoglobin, when compared to the diabetic control group [G-II].

Effect of Methanolic extract of *Diospyros ferrea* leaf on liver glycogen levels in streptozotocin induced diabetic rats shown in the Table-6. In group treated with streptozotocin [G-II] [60 mg/Kg, i.p., single dose] a significant depletion in liver glycogen levels on 21st day was observed when compared to the normal group [G-I]. Treatment with standard drug [Glibenclamide, 0.5 mg/Kg, p.o., once daily] significantly enhances liver glycogen levels, when compared to the diabetic control [G-II]. Treatment with MEDFL [200 and 400 mg/Kg] to group IV and V does not produce significant effect on liver glycogen levels when compared to the diabetic control [G-II].

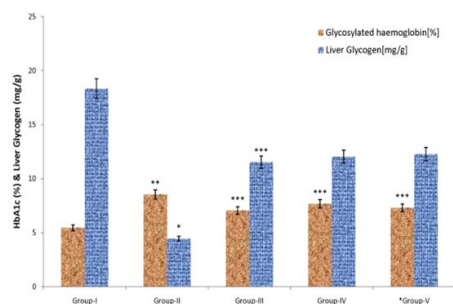


Fig:5 Effect of MEDFL on Glycosylated Haemoglobin and Liver Glycogen

Table-6: Effect of MEDFL on Glycosylated Haemoglobin and Liver Glycogen

Group	Treatment	On 21 st Day	
		Glycosylated haemoglobin[%]	Liver Glycogen[mg/g]
Group-I	Normal	5.453±1.199	18.333±2.883
Group-II	Diabetic Control [60 mg/Kg, i. p]	8.540±1.307 ^{**}	4.460±0.930 [*]
Group-III	Glibenclamide [0.5mg/Kg p. o.]	7.053±1.646 ^{***}	11.533±0.895 ^{***}
Group-IV	MEDFL [200mg/Kg p. o.]	7.679±1.249 ^{***}	12.036±1.322
*Group-V	MEDFL [400mg/Kg p. o.]	7.299±1.717 ^{***}	12.275±1.416

All values were expressed as Mean ± SEM. n=6
^{***} means p<0.001, ^{**} means p<0.01, ⁺ means p<0.05 when compared with Normal Control.
^{***} means p<0.001, ^{**} means p<0.01, ⁺ means p<0.05 when compared with diabetic control

Effect of Methanolic extract of *Diospyros ferrea* leaf on Liver antioxidant enzyme levels in Streptozotocin induced diabetic rats shown in the Table-7 and Fig-6.

A significant increase in the levels of LPO was observed in the diabetic control group, when compared to the normal group [G-I]. The group-III receiving standard drug had significant decrease in the LPO levels, when compared to the diabetic control group [G-II]. The groups-IV and V treated with MEDFL [200 and 400 mg/Kg] also exhibited a significant decrease in the LPO levels, when compared to the diabetic control group [G-II].

A significant decrease in the levels of SOD was observed in the diabetic control group, when compared to the normal group [G-I]. The group-III receiving standard drug had significant increase in the SOD levels, when compared to the diabetic control group [G-II]. The groups-IV and V treated with MEDFL [200 and 400 mg/Kg] also exhibited a significant increase in the SOD levels, when compared to the diabetic control group [G-II].

A significant decrease in the levels of Catalase was observed in the diabetic control group, when compared to the normal group [G-I]. The group-III receiving standard drug had significant increase in the catalase levels, when compared to the diabetic control group [G-II]. The groups-IV and V treated with MEDFL [200 and 400 mg/Kg] also exhibited a significant increase in the catalase levels, when compared to the diabetic control group [G-II].

A significant decrease in GSH levels was observed in the diabetic control group, when compared to the normal group [G-I]. The group-III receiving standard drug had significant increase in the pancreatic GSH levels, when compared to the diabetic control group [G-II]. The groups-IV and V treated with MEDFL [200 and 400 mg/Kg] also exhibited a significant increase in the pancreatic GSH levels, when compared to the diabetic control group [G-II].

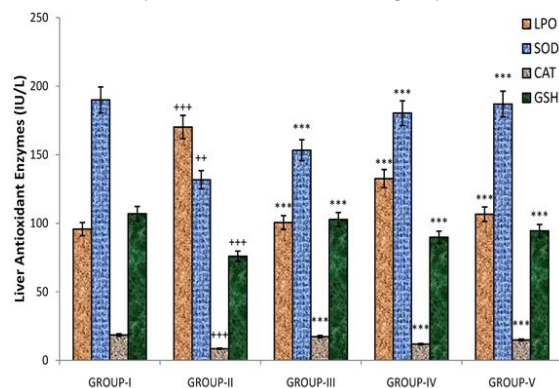


Fig 6: Effect of MEDFL on Liver Antioxidant Enzymes

Table-7: Effect of MEDFL on Liver Antioxidant Enzymes

Group	Treatment	Liver Antioxidant Enzymes			
		LPO [IU/L]	SOD [IU/L]	CAT [IU/L]	GSH [IU/L]
GROUP-I	Normal	95.83 ± 1.537	190.5±1.18	18.5 ± 0.428	107 ± 1.528
GROUP-II	Diabetic Control [60 mg/Kg, i. p]	170.166 ± 2.822 ⁺⁺⁺	131.83±0.945 ⁺⁺⁺	8.5 ± 0.428 ⁺⁺⁺	76 ± 1.291 ⁺⁺⁺
GROUP-III	Glibenclamide [0.5 mg/Kg p. o.]	100.66 ± 0.714 ^{***}	187.01±1.065 ^{***}	17.33 ± 0.333 ^{***}	102.83 ± 1.078 ^{***}
GROUP-IV	MEDFL[200 mg/Kg p. o.]	132.66 ± 1.145 ^{***}	153.33±1.498 ^{***}	11.83 ± 0.307 ^{***}	89.83 ± 1.302 ^{***}
GROUP-V	MEDFL [400 mg/Kg p.o.]	106.66 ± 1.145 ^{***}	180.33±1.282 ^{***}	14.83 ± 0.307 ^{***}	94.5 ± 0.670 ^{***}

All values were expressed as Mean ± SEM. n=6

+++ means $p < 0.001$, ++ means $p < 0.01$, + means $p < 0.05$ when compared with Normal Control.

+++ means $p < 0.001$, ++ means $p < 0.01$, + means $p < 0.05$ when compared with diabetic control

Discussion

Streptozotocin induced diabetic rats exhibited significant increase in blood glucose level. So Chronic treatment with methanolic extract of *Diospyros ferrea* leaf reduced blood glucose level throughout the experimental period in duration dependent manner indicating its anti-hyperglycemic activity. However blood glucose levels were not altered in normoglycemic rats further strengthening the anti-diabetogenic potential of the extract. A significant decrease in total cholesterol was determined by the oral administration of the MEDFL and a slight decrease was observed in animals treated. The results indicate the possibility that *Diospyros ferrea* leaf could be effective to prevent or retard the development of diabetes complications due to metabolic disorders. Cholesterol and triglyceride levels of serum were observed lowered near to normal in treated rats. However no significant change in urea, uric acid and creatinine levels. In diabetic rats glycosylated haemoglobin was observed increased and decreased in treated rats.

Investigation on the effects of administration of the MEDFL on the serum aspartate aminotransferase [AST] and alanine aminotransferase [ALT] revealed that acute treatments for 21st days with the plant extract resulted in a sharp rise in the serum enzyme levels. A significant decrease in the activity of antioxidant enzymes viz., Catalase, reduced glutathione and lipid peroxidase were observed in the blood serum. However there was a significant increase in liver glycogen in rats treated with different doses of plant extracts. It is noted that discrete changes in pancreas which confirm favourable development after four weeks of treatment and beneficial role of administration of these herbal remedies. From the above discussion it is concluded that methanolic leaf extract of *Diospyros ferrea* leaf at high dose (400 mg/kg) exhibited significant antihyperglycemic activity than plant leaf extract at low dose (200 mg/kg) in Streptozotocin-induced diabetic rats. Further investigation is necessary to determine the exact phytoconstituent (s) which are responsible for antidiabetic effect.

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