



ANTI-HYPERLIPIDEMIC EFFECT OF ETHANOLIC LEAF EXTRACT OF *GMELINA ARBOREA* IN STREPTOZOTOCIN INDUCED MALE WISTAR ALBINO RATS

DAVID PUNITHA¹, ANGAMUTHU THANDAVAMOORTHY, KARUPPANAN ARUMUGASAMY^{2*}, SREENIVASAPURAM NATARAJAN SURESH³, UTHAMAN DANYA² AND MADATHUPATTI RAMANATHAN UDHAYASANKAR²

¹Department of Botany, Providence College for women, Coonoor, The Nilgiris, India.

^{2,3}Department of Botany and Biotechnology, Kongunadu arts and science college (Autonomous), Coimbatore, Tamilnadu, India.

* Corresponding Author email: arumugasamy_kasc@yahoo.co.in

ABSTRACT

The antihyperlipidemic effects of ethanolic leaf extract of *Gmelina arborea* (Verbenaceae) in male wistar albino rats was evaluated. The ethanolic extract of *G. arborea* at a dose of 150 mg/kg of body weight and standard drug glibenclamide at the dose of 100µg/kg given to the animal models. The extract exhibited significant hypoglycemic activity in animal models when compared with a standard antidiabetic drug Glibenclamide. The hypoglycemia produced by the extract may be due to increased uptake of glucose at tissue level and or increase in pancreatic β -cell function or due to inhibition of intestinal glucose absorption of glucose. The lipid profile such as TC, TG and LDL levels were significantly increased in diabetic control animals where as HDL levels were decreased when compared to the control rats. The significant changes were also occurred in the rats treated with ethanolic *G. arborea* extract. The findings of the present study suggest that the ethanolic extract of *G. arborea* produced significant antihyperglycemic activity in STZ induced diabetic rat which is comparable to that the Glibenclamide.

Key Words: *G. arborea*, Diabetes mellitus, antihyperlipidemia, Streptozotocin.

1. INTRODUCTION

Diabetes mellitus is a clinical syndrome characterized by inappropriate hyperglycemia caused by a relative or absolute deficiency of insulin or by a resistance to the action of insulin or by a resistance to the action of insulin at the cellular level. It is the most common endocrine disorder, affecting 200 million individuals in the world wide (Debra 1991). Apart from the currently available therapeutic options, many herbal medicines have been recommended for the treatment of diabetes. Herbal drugs are prescribed widely because of their

effectiveness, less side effects and relatively low cost.

Gmelina arborea belongs to family Verbenaceae a plant of Indian origin having tremendous therapeutic potentials. It is commonly known as Kumla in tamil and Gamhar in Hindi. It is a fast growing species and known to have been used in traditional Indian medicine. The bark of *G. arborea* are stomachic, galactagogue, laxative and anthelmintic, improve appetite, useful in hallucination, piles, abdominal pains, burning

sensations, fever, 'Tridosha' and urinary discharge. It is also recommended with other drugs for the treatment of snake-bite and scorpion-sting. Leaf paste is applied to relieve headache and juice is used as wash for ulcers. Gamhar leaves, Apamarga roots and bark skin of Saimali are mashed with cow's milk and are given orally to treat hyperacidity. The leaves juice, milk and sugar are recommended in inflammatory condition of urinary bladder and dysuria. Almost all parts of this tree are used in folk medicine for treating various stomach disorders, fevers and skin problems (Sharma et al, 2001). The plant extracts are reported to exhibit anti-inflammatory and wound healing properties (Shirwaikar et al, 2003) and are also known to inhibit platelet aggregation (Faiza and Darakhshanda, 1998). Chemical constituents of *Gmelina* include lignans (Anjaneyulu et al, 1977), flavonoids (Nair and Subramanian, 1975), iridoid and phenylpropanoid glycosides (Hosny and Rosazza, 1998) and an isoxazole alkaloid (Barik et al, 1992).

2. MATERIALS AND METHODS

2.1. COLLECTION OF PLANT MATERIAL:

The leaf of *Gmelina arborea* was collected during blooming season (November, 2010) from nearby sholas of Kothagiri Hills the Nilgiri District, Western Ghats, Southern India, Tamil Nadu. The plants were identified and authenticated by a plant taxonomist.

2.2. PREPARATION OF EXTRACT:

250 g of collected samples were washed 2-3 times with water followed by distilled water and shade dried. The dried parts of plants were pulverized by mechanical grinder (Willey mill) to get the powder through 100 mesh sieve and then stored in a desiccator. The shade dried and powdered plant materials were extracted with petroleum ether to remove the resins and the residue was then extracted with methanol by using soxhlet apparatus.

2.3. EXPERIMENTAL ANIMALS AND STZ-INDUCTION OF DIABETES

Male Wistar Albino rats weighing 180-250 g were obtained from the animal house of the laboratory of Agricultural University, Trissur, Kerala. The rats

were housed in polycarbonated cages at a temperature regulated (22°C) and humidity (55%) controlled room with a 12 h light/12 h dark cycle, water and standard pellet diet were available to the animals throughout the experimental period. The experimental protocol has been approved by the Institution Animal Ethics committee and by the Regulatory body of the government (659/02/a/CPCSEA). The rats were injected intraperitoneally with STZ monohydrate dissolved in sterile 9% saline at a dose of 200 mg kg⁻¹ b.wt. Two weeks after the induction, moderate diabetes having hyperlipidemia and hyperglycemia (i.e. with a blood glucose of 200-300 mg dL⁻¹) were observed in the rats.

2.4. DRUGS AND REAGENTS

Drugs and fine chemicals were purchased from Sigma-Aldrich, Mumbai. All preparations were freshly made in distilled water prior to the experiments.

2.5. EXPERIMENTAL DESIGN

The STZ induced rats were divided into four groups each contains five numbers of rats. Group I served as normal rats without any induction and treatment, Group II served as STZ (200 mg/kg b.wt.) induced diabetic control rats. Group III served as diabetic rats given ethanolic leaf extract of *Gmelina arborea* (150 mg/kg b.wt.). Group IV diabetic rats given glibenclamide (100mg/kg b.wt.)

The animals were carefully monitored every day and weighed every week (2 weeks). No sign of toxicity was noticed on the behaviour and general health of the animals when exposed to extract. Animals described as fasted were deprived of food for at least 12 h but allowed free access to drinking water. Blood samples were drawn at end of study. Blood glucose estimation, body weight, food and water intake measurement were done on 14th day of the study. On day 14, rats were sacrificed by cervical dislocation under ether anesthesia. Blood was collected from overnight fasted rats, allowed to clot and centrifuged at 3000 rpm for 15 minutes. Serum samples were separated and used for biochemical analysis. The samples were stored at -80° C, if not used immediately.

2.6. STATISTICAL ANALYSIS

All data were expressed as means \pm S.E. Significant differences among the groups were determined by one-way analysis of variance using the DMRT statistical analysis program. Statistical significance was considered at $p < 0.05$.

3. RESULTS

The hyperlipidemia and hyperglycemia of the plant extract were assessed on albino rats using STZ as toxicant. The table 1 and figure 1 illustrated that the effect of *G. arborea* on serum fasting glucose level in diabetic rats. The results show that the extract

caused reduction in blood glucose level as (81 ± 2.5 mg/dl) when compared to STZ induced rats (200 ± 11.80 mg/dl). Ethanolic leaf extract caused significant ($p < 0.001$) decrease in the fasting glucose. However, diabetic rats treated with glibenclamide a standard drug has exhibited maximum reduction in fasting blood sugar (77 ± 4.50 mg/dl) is compared to normal (75 ± 4.56 mg/dl). Whereas the rat treated with STZ showed (200 ± 11.80 mg/dl) more blood glucose as compared with normal extract and glibenclamide treated groups. The qualitative glucose analysis in groups has exhibited less glucose levels as compared to STZ induced group, except normal/untreated group.

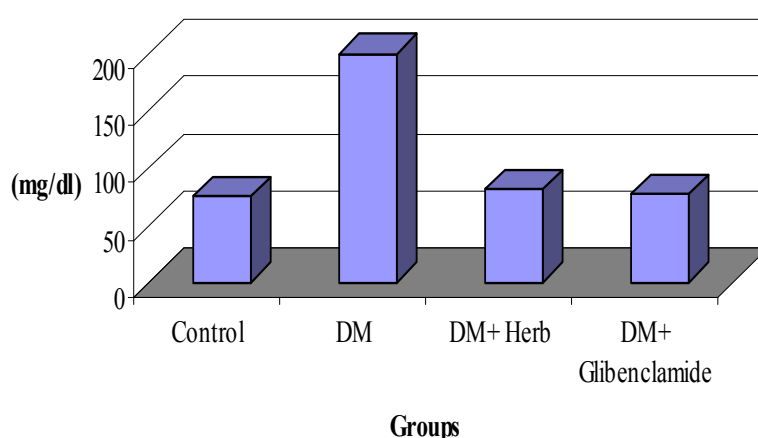
Table (1) The effect of ethanolic leaf extract of *Gmelina arborea* on blood glucose.

Groups	Fasting blood glucose (mg/dl)
Group I- Normal control	75 ± 4.56
Group II- Diabetic control	$200 \pm 11.8^*$
Group III- Diabetic + <i>G. arborea</i>	$81 \pm 2.5^{**}$
Group IV- Diabetic + Glibenclamide	$77 \pm 4.5^{**}$

Values are mean \pm of $n=6$ rats each group

Values significant at $*p < 0.001$ as compared to normal, $**p < 0.001$ as compared with Diabetic control, NS= not significant

Fig 1. The effect of ethanolic leaf extract of *Gmelina arborea* on Fasting blood sugar



The lipid profile such as TC, TG and LDL levels were significantly increased in diabetic control

animals (DC) where as HDL levels were decreased when compared to the control rats. The plant extract

was administered orally at the dose levels of 400mg/kg b.wt. to diabetic rats. The diabetic animals at 400mg/kg dosage recorded a significant change in the TC, TG, LDL and HDL levels. And also a significant ($P<0.001$) depletion in the total cholesterol level was recorded in the diabetic animals. The depletion in the TC, TG and LDL were done at the dose dependent manner and the highest

reduction in the cholesterol recorded were 170 ± 11.5 mg/dl, TG (102 ± 4.8 mg/dl), LDL (16.7 ± 3.3) and HDL (41.5 ± 2.1 mg/dl) when compare to the diabetic control animals. The depleted high density lipoprotein (HDL) in the diabetic rats, increased significantly ($P<0.001$) after the administration of the plant extract.

4. DISCUSSION

In diabetes, the increased blood sugar levels might be due to either insulin resistance of the body cells or decreased secretion of insulin from beta cells manifest in the decreased serum insulin levels (Mohammad Ali et al, 2004). The reduction in the serum insulin levels in the STZ treated rats might be attributed to the reduced secretion of the hormone which might be due to the damage of the beta cells of endocrine pancreas. The STZ selectively destroys the pancreatic cells and induce hyperglycemia (Kurup and Bhonde, 2000). Similar studies were recorded earlier in the STZ treated rats, the levels of serum insulin significantly reduced (Yoon and Ray, 1985). Nitric oxide has been demonstrated to participate in the beta cell damage during STZ induced diabetes (Duran Reges et al, 2004).

Diabetes affects both glucose and lipid metabolism (Sperling et al, 2000). In the post prandial state elevated serum insulin increases lipoprotein lipase activity in adipose tissue and promotes fuel storage as triglycerides in normal metabolism (Bhagavan, 2002). The insulin deficiency depletes the activity level of lipoprotein lipase, thus leading to deranged lipoprotein metabolism during diabetes (Ranganathan et al, 2000). The lipoprotein levels in the STZ induced diabetic rats of the present study reveal a significant alter in lipoprotein metabolism. The serum total cholesterol content increased significantly in diabetic animals. The elevated hypertriglyceridemia was increased in the synthesis of triglyceride rich lipoprotein particles in liver diminished catabolism

in diabetic rats (Ginsberg, 1991). Since insulin has a potent inhibitory effect on lipolysis in adipocytes, insulin deficiency is associated with excess lipolysis and increased influx of free fatty acids to the liver (Ohno, 2000). The increased levels of low-density lipoprotein (LDL) in the diabetic animals might be due to over production of LDL by the liver due to the stimulation of hepatic triglyceride synthesis as a result of free fatty acid influx (Coppack, 1994). The high density lipoprotein (HDL) was significantly reduced in the diabetic rats which indicate a positive risk factor for atherosclerosis (Bopanna, 1997). After the administration of the combined aqueous extract to the STZ induced diabetic rats revealed augmented serum insulin levels. The increment of serum insulin levels might be due to increased secretion of the hormone, which might reflect the probable 'repair' of the damaged beta cells of the endocrine of the pancreas due to STZ.

The blood glucose level of plant extract fed animal was significantly ($P<0.001$) reduced. The highest depletion was recorded in the 400mg/kg body wt., dosage rats. The levels of serum TC, TG and LDL were found to be significantly reduced in the plant extract treated diabetic animals. This might be due to the reduced hepatic triglyceride synthesis and or reduced lipolysis that might be due to the increase in serum insulin levels in the plant extract treated rats. The HDL increased significantly in the plant extract treated rats indicating a reversed atherogenic risk.

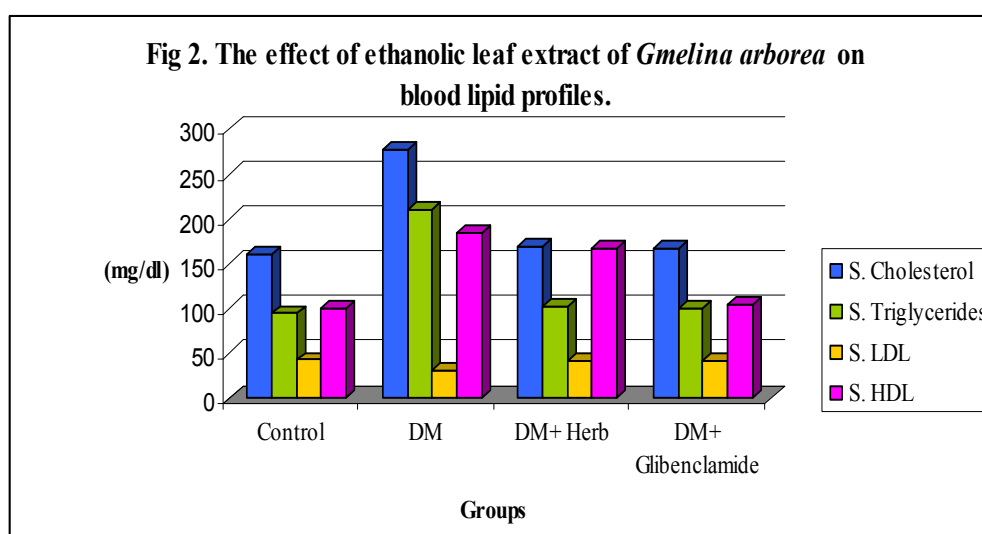
Table (2) The effect of ethanolic leaf extract of *Gmelina arborea* on blood lipid profile.

Groups	Drug	Serum Cholesterol (mg/dl)	Serum Triglycerides (mg/dl)	Serum LDL (mg/dl)	Serum HDL (mg/dl)
Group I- Normal control	Saline	160 ± 7.8	95 ± 4.5	43 ± 2.4	100 ± 5.9
Group II- Diabetic control	200 mg/kg b.wt. STZ	278 ± 0.12*	210 ± 9.5*	31 ± 3.5**	185 ± 9.5
Group III- Diabetic + <i>G. arborea</i>	150 mg/kg b.wt.	170 ± 11.5**	102 ± 48**	41.5 ± 2.1**	167 ± 3.3**
Group IV- Diabetic + Glibenclamide	100 mg/kg b.wt.	168 ± 7.7**	100 ± 5.9**	42 ± 3.7**	105 ± 6.2**

Values are mean ± of n=6 rats each group

Values significant at *p<0.001 as compared to normal, **p<0.001 as compared with Diabetic control,

NS= not significant



5. CONCLUSION

The potency of herbal drugs is significant and they have negligible side effects than the synthetic antidiabetic drugs. There is increasing demand by patients to use the natural products with antidiabetic activity. In recent times there has been renewed interest in the plant remedies. Plants hold definite

promises in the management of Diabetes mellitus. Isolation and identification of active constituents from the plant, preparation of standardized dose and dosage regimen can play a significant role in improving the hypoglycaemic action.

6. REFERENCES

1. Anjaneyulu ASR, Rao AM, Rao VK, Row LR, Pelter A and Ward RS. Novel hydroxy lignans from the heartwood of *Gmelina arborea*. *Tetrahedron*. 1977; 33: 133–143.
2. Barik BR, Bhowmik T, Dey AK, Patra A, Chatterjee A and Joy SS. Premnazole an isoxazole alkaloid of *Premna integrifolia* and *Gmelina arborea* with anti-inflammatory activity. *Fitoterapia*. 1992; 63 (4): 295–299.
3. Bhagavan NV. Metabolic homeostasis In: N.V. Bhagavan (Eds) *Medical biochemistry Harcourt/Academic, Florida*. 2002, p. 485–519.
4. Bopanna KN, Kannan J, Sushma gangil, Blaraman R, Rathod SP. Antidiabetic and anti hyperlipedemic effect of Neem, Lipedemic effect of Neem seed kernel powder on alloxon diabetic rabbits. *Ind. J. Pharmacology*. 1997; 29: 162–167.
5. Coppack SW, Jenson MD, Miles JM. *In vivo* regulation of lipolysis in human. *J. Lipid Res*. 1994; 35:177–193.
6. Debra HJ. Management of diabetes mellitus perspective of care across the life span, 2nd edition, Haven press, New york, 1991, Vol I, p. 345–54.
7. Duran Reges G, Pascoe Lira D, Vilar Rojas C, Medina Navarro R. Diabetogenic effect of STZ diminishes with loss of Nitric oxide role of ultra violet and carboxy PT10. *Pharmacology*. 2004; 71(1):17–24.
8. Faiza H and Darakhshanda S. The inhibition of platelet aggregation and related physiological responses with crude drug extract of *Gmelina arborea*, In: Sixth International Symposium on New trends in Natural Products Chemistry, Publication 1998, p. 279–86.
9. Ginsberg HN. Lipoprotein physiology in nondiabetic and diabetic states. Relationship to atherogenesis. *Diabetes care*. 1991; 14: 839– 855.
10. Hosny M and Rosazza PN. Gmelinosides, Twelve acylated iridoid glycosides from *Gmelina arborea*. *J. Nat. Prod*. 1998; 61: 734–742.
11. Kurup S and Bhonde RR. Combined effect of nicotinamide and streptozotocin on diabetic status in partially pancreatectomized adult BALB/C mice. *Horm Metab Res*. 2000; 32(8):330–334.
12. Mohammad Ali E and Razeih Y. Hypoglycemic effect of *Teucrium polium* studies with rat pancreatic islets. *J. Ethanopharmacol*. 2004; 95: 27–30.
13. Nair AGR and Subramanian SS. Quercetagenin and other flavones from *Gmelina arborea* and *G. asiatica*. *Phytochemistry*. 1975; 14: 1135–1136.
14. Ohno T, Horio F, Tanaka S, Terada M, Namikawa T and Kitoh J. Fatty liver and hyperlipidemia in IDDM of streptozotocin treated shrews. *Life Sci*. 2000; 66:125–131.
15. Ranganathan G, Li C, Kern PA. The translational regulation of lipoprotein lipase in diabetic rats involves the 3'-untranslated region of lipoprotein lipase mRNA. *J.Biol.Chem*. 2000; 275:40986–40991.
16. Sharma PC, Yelne MB and Dennis TJ. Database on Medicinal Plants Used in Ayurveda, vol. 3, Central Council for Research in Ayurveda and Siddha, Department of ISM and H Ministry of health and Family Welfare Government of India, 2001, p. 217–228.
17. Shirwaikar A, Ghosh S and Rao PGM. Effect of *Gmelina arborea* Roxb. leaves on wound healing in rats. *J. Nat. Remedies*. 2003; 45–48.
18. Sperling MA and Saunders PA. Diabetes mellitus In: R.E. Behrman R.M, Kliegman H.B, Jenson (Eds) Nelson text book of pediatrics, 2000, p.1767–1791.
19. Yoon JW and Ray UR. Perspectives on the role of viruses in insulin dependent diabetes. *Diabetes care*. 1985; 8(1):39–44.