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Antidiabetic property of *Embelia tsjeriam-cottam* plant leaf extract in rats

Dr. Mathen Melba Sara	MVSc Scholar, Department of Veterinary Pharmacology and Toxicology,Veterinary College, Bangalore, Karnataka Veterinary, Animal and Fisheries Sciences University, Bidar
DR. N.B. Shridhar	Associate Professor, Department of Veterinary Pharmacology and Toxicology, Veterinary College, Bangalore, Karnataka Veterinary, Animal and Fisheries Sciences University, Bidar
Dr. Jagadeesh S. Sanganal	Head and Associate Professor, Department of Veterinary Pharmacology and Toxicology, Veterinary College, Bangalore, Karnataka Veterinary, Animal and Fisheries Sciences University, Bidar
Dr. Suguna Rao	Professor, Department of Veterinary Pathology, Veterinary College, Bangalore, Karnataka Veterinary, Animal and Fisheries Sciences University, Bidar
Dr. Rekha Yadav	MVSc Scholar, Department of Veterinary Pharmacology and Toxicology, Veterinary College, Bangalore, Karnataka Veterinary, Animal and Fisheries Sciences University, Bidar

ABSTRACT

The Embelia tsjeriam-cottam leaf extract was evaluated for antidiabetic property in Wistar rats. The various groups in the present study included normal control (Group I), diabetic control (Group II), glibencamide treated (Group III), leaf extract treated groups (Group IV, V and VI).Diabetes was induced in rats by streptozotocin and the diabetic rats treated with leaf extract produced significant improvement in glucose, cholesterol and triglycerides, but the antidiabetic effect of leaf extract treated group was lesser than the glibenclamide treated group. The results obtained were also supported by the histopathological evaluation

KEYWORDS : Embelia tsjeriam-cottam; Diabetes; Streptozotocin

INTRODUCTION

Embelia (Family: Myrsinaceae) has been reported to have great pharmacological potential with a great utility and usage as folklore medicine. Embelin, the main active principle of the plant is reported to possess many pharmacological properties including antidiabetic, anthyperlipidemic and antioxidant properties (14). *Embelia ribes* (Vidanga) has anti-fertility, analgesic, antibacterial, anti-inflammatory, antioxidant, cardio protective and anti-diabetic activity (12), antihelminthic (10, 11), anti bacterial activity (7), anti fungal activity (15), amylase inhibitory activity, trypsin inhibition, adaptogenic activity, anti cancer activity, anticonvulsant activity, wound healing, anti hyperlipidemic activity (2) etc. The fruits of *Embelia tsjeriam-cottam* (Vaivilanga) are being widely used in place of *Embelia ribes.*

Diabetes mellitus is a heterogeneous group of disorder characterized by chronic hyperglycemia, polyurea, polydipsia, polyphagia, emaciation, and weakness due to disturbance in carbohydrate, fat, and protein metabolism, and directly related to absolute or relative deficiency in insulin secretion and/or insulin action (8). Oral administration of *E. ribes* ethanolic extract in dose of 100 mg/kg and 200 mg/kg significantly reduced the levels of blood glucose, glycated haemoglobin, heart rate and systolic blood pressure in streptozotocin induced diabetic rats when compared with diabetic control group and chronic administration of *E. ribes* ethanolic extract at the dose of 200 mg/kg has shown a significant reduction in systolic blood pressure and heart rate, thus, providing protection in diabetes (4). Embelin isolated from the berries of *Embelia basal* reduced the elevated plasma glucose, lipid profile, ameliorated oxidative stress, and inhibited intracellular proinflammatory mediators in diabetic rats, indicating that pro-inflammatory mediators and oxidative stress may be major triggering factors in type 2 diabetes mellitus (13). Embelin, isolated from *Embelia ribes* fruit, exhibited insulin sensitizing effect through adipose tissue specific partial agonism of PPARγ (peroxisome proliferator-activated receptors) and activated glucose transport through translocation and activation of GLUT4 mediated by insulin dependent in epididymal adipose tissue and also protected beta-cells by scavenging free radicals and alleviated dyslipidemia in insulin resistant animal model (9). *Embelia ribes* ethanol extract showed a preventive effect on body weight gain, visceral fat accumulation and elevated blood pressure. The extract treatment elicited a significant reduction in serum levels of leptin by 45%, insulin by 37%, glucose by 28%, total cholesterol by 18%, and triglycerides by 24% while HDL level increased by 31% (5). Hence, the present study was designed to study the antidiabetic property of *Embelia tsjeriam-cottam* plant leaf extract in rats.

MATERIALS AND METHODS

Plant material: *E. tsjeriam-cottam* fresh leaves were collected from Shimoga District of Karnataka State during the month of october, 2013. The *E. tsjeriam-cottam* leaves were dried under shade and were finely powdered. Methanolic extract was prepared from whole leaf powder.

The study was conducted in diabetic induced male Wistar albino rats weighing 150 to 200 g for 28 days. They were divided into six groups of ten male rats in each group. Group I animals served as the normal group. The other animals were fasted overnight and diabetes was induced by a single intraperitoenal injection of freshly prepared solution of streptozotocin STZ (45 mg/kg) in 0.1 M cold citrate buffer having a pH 4.5. The diabetic state induced was confirmed by estimating the blood glucose levels after 72 h of STZ. The animals that showed the blood glucose level above 200 mg/dl were considered as diabetic. Group II animals were diabetes induced and served as the diabetic control group which were administered orally with normal saline. Glibenclamide an oral hypoglycemic drug was used as a reference drug (Group III) and was administered orally at the dose of 600 µg/kg/ day. The leaf extract was gavaged to the diabetic rats of group IV,

V and VI with respective dose of 100, 200, 300 mg/kg.

To evaluate the biochemical parameters, serum was collected for which blood was drawn from the retro-orbital plexus on day 7, 14, 21 and 28 of post STZ injection of the study. The serum was analyzed for serum glucose, cholesterol and triglycerides. Two rats from each group were sacrificed under anaesthesia on day 7, 14 and 21 and the remaining rats on day 28 of the experiment and were subjected for detailed post mortem examination and gross changes, if any were recorded. Further representative tissue samples from pancreas was collected in 10 % neutral buffered formalin (NBF) for the histopathological evaluation. The data were analysed by Two way analysis of variance (ANOVA).

RESULTS AND DISCUSSION

Table 1: The biochemical parameters on day 28

Details	serum glucose (mg/dl)	serum cholesterol (mg/dl)	serum triglycerides (mg/dl)
Group I	105.58±0.97	40.97±1.07	97.90±0.56
Group I I	418.95±3.45	117.13±0.46	326.05±5.46
Group I I I	187.77±2.59	34.18±0.75	89.22±0.53
Group I V	258.28±3.51	43.30±0.81	102.97±0.48
Group V	243.27±4.18	42.61±0.91	102.46±0.86
Group VI	252.87±2.80	40.70±0.53	100.85±0.86

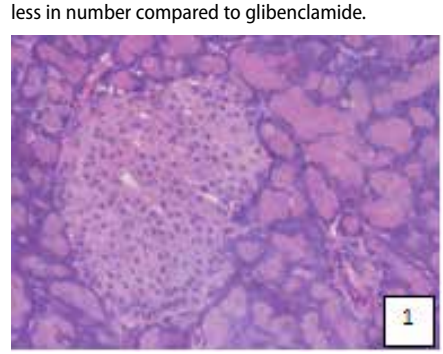
Values are expressed as Mean ± SE, n=6

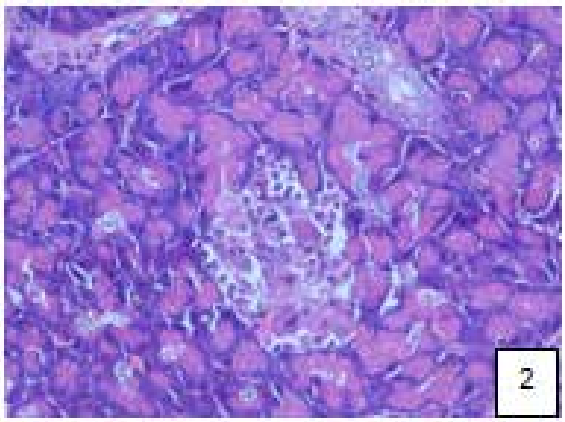
The diabetic animals attained a blood glucose level of more than 200 mg/dl, 72 h post STZ injection. This finding is in accordance with earlier findings (17). The mean fasting serum glucose values of Group II were significantly higher (P<0.001) as compared with those of normal control animals and glibenclamide treated animals. The mean serum glucose levels in the diabetic rats treated with glibenclamide were significantly lower (P<0.001) from those of diabetic control group rats. The serum glucose levels (mg/dl) in Group IV rats gavaged with leaf extract 100 mg/kg on day 28 were found significantly higher (P<0.001) compared to normal control animals and with respect to glibenclamide, but the glucose levels showed a decrease on day 28. In Group V, level was significantly low (P<0.001) on day 28 compared to diabetic control group values but significantly higher (P<0.001) in comparison with those of normal control rats and with respect to glibenclamide treated rats. The mean serum glucose levels in the Group VI showed a significant decrease (P<0.001) in the serum glucose level on day 28 compared to diabetic control group values. But when compared to the normal control group they showed a significant increase (P<0.001) in serum glucose level throughout the study. In addition compared with glibenclamide treated group the serum values were higher. The leaf extract treated groups (IV, V and VI) showed a significant (P<0.001) decrease in the serum glucose level in day 28, but the values were significantly high (P<0.001) when compared to glibenclamide. A significant decrease in glucose level of STZ administered rats fed with *Embelia ribes* fruit extract for 40 days was observed before also (3). Embelin could improve adipose tissue insulin sensitivity without increasing weight gain, enhance glycemic control and protect β-cell from damage (9).

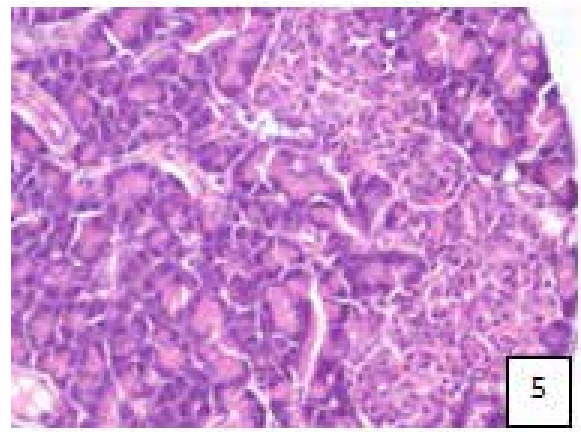
The diabetic rats of Group II revealed a significant increase (P<0.001) in the serum cholesterol level from day 1 to 28 day of experiment in comparison with that of normal control. After induction of diabetes, increased in serum cholesterol concentration was observed (16). Cholesterol concentration of all the groups treated has shown a decrease as compared to diabetic control on day 28. Administration of glibenclamide showed significant reduction in the cholesterol level. The serum cholesterol level (mg/dl) in Group III rats were significantly lower (P<0.001) on day 28 compared to diabetic control group rats. The serum cholesterol level (mg/dl) in Group IV, V and VI rats on day 28 were significantly lower (P<0.001) when compared to diabetic control group and significantly higher (P<0.001) to glibenclamide treated group on day 28. Similar to the above finding, Embelin at a dose rate of 50 mg/ kg produced a significant (P<0.01) decrease in total cholesterol (23.29%), triglyceride (53.31%) and free fatty acid (41.35%) in diabetic rats compared to diabetic control rats (9).

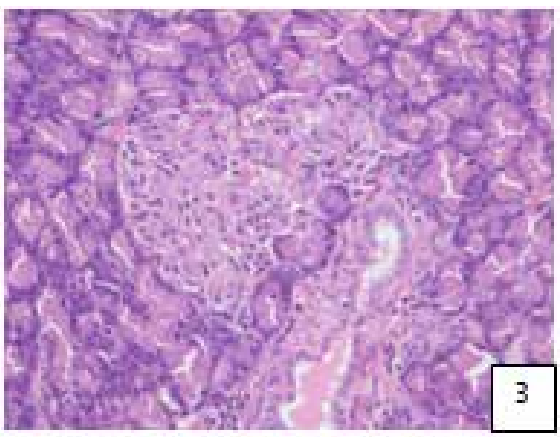
There was a marked increase in serum triglyceride level in diabetic animals over control group. This is in accordance with the finding of others (1). In streptozotocin-induced diabetes there is excess of fatty acids in the serum, which are converted into phospholipids and cholesterol in liver (6). These two substances along with excess triglycerides formed at the same time in liver might be discharged into blood in the form of lipoproteins. The abnormal high concentration of serum lipids in the diabetic subject might be mainly due to increase in the mobilization of free fatty acids from the peripheral fat depots, since insulin inhibits the hormone sensitive lipase. The diabetic rats of Group II revealed a significant increase (P<0.001) in the serum triglyceride levels when compared with those of normal control animals. The serum triglyceride level (mg/dl) in Group III rats were significantly low (P<0.001) on day 28 day compared to diabetic control group. The serum triglyceride level (mg/dl) in Group IV, V and VI rats were significantly low (P<0.001) on day 28 day compared to diabetic control group values but significantly higher (P<0.001) than glibenclamide group. There was no significant (P>0.05) increase in the serum triglyceride level (mg/dl) of Group IV, V and VI on day 28 compared to normal control group. The serum triglyceride level of all the groups treated showed a decrease as compared to diabetic control. Administration of glibenclamide showed significant reduction in the triglyceride level. This is in accordance with earlier findings who described a decrease in serum triglyceride level of diabetic rats treated with embelin (9). A decrease in the serum triglyceride, very low density lipid and low density lipid in STZ induced HFD diabetic rats on treating with embelin was reported earlier (13). They also reported tht embelin has a stimulatory effect on the pancreatic beta cells to secrete insulin.

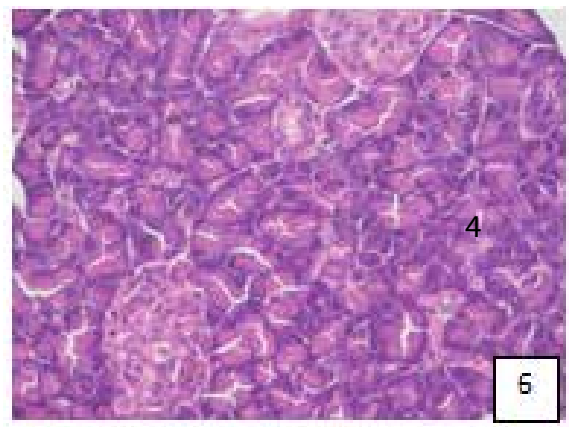
In the histopathological study on day 28, pancreas of normal control animals (Group I) was normal in appearance of acini and islets of Langerhans. Group II rats revealed persistence of STZ induced effect on pancreas. They showed destruction of β-cells, degeneration and necrosis of β-cells. The decrease in cellularity within islets of Langerhan's was observed. There was some degree of regeneration involving the formation of multiple pancreatic lobules. No well formed islets were observed. The pancreas of standard oral drug glibencamide treated rats (Group III) revealed regeneration and proliferation of β-cells. Group III showed a marked improvement in the architecture with increased cellularity. More number of islets were seen and the islets were placed close to the pancreatic duct. The islets were consisted of well formed β-cells with cytoplasmic granularity. Hyperplastic changes were also observed with pancreatic duct along with compact arrangement of normal appearing exocrine portion. Group IV on day 28 also revealed formation of new islets though less in number. The newly formed islets were small in size. The exocrine portion showed small sized acini. Group V showed irregularly shaped newly formed islets along with normal appearing architecture. The islets showed hyper cellularity. The hyperplastic islets were seen extending to the surrounding exocrine portion. The exocrine portion also showed a compact arrangement. The rats of Group VI on day 28 has newly formed islets along with nomal architecture of β-cells. Adjacent to the islets duct hyperplasia was seen. There was overall improvement in the cellularity but persistence of STZ effect was present in all the three extract treated groups. The leaf extract treated groups (IV, V and VI) showed irregularly shaped newly formed islets along with hyper cellularity. Histology of the pancreatic tissues in embelin (50 mg/kg) treated diabetic groups showed regranulation and protective effect on β-cells (9). Similar results were observed in other studies also (3). But the islets formed in the leaf extract treated groups were small and less in number compared to glibenclamide.











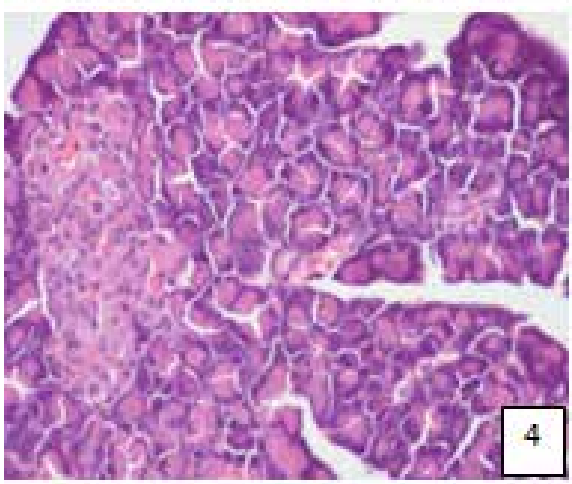


Figure: Sections of the pancreas showing 1) Normal architecture of Islet of Langerhans in normal control group.
2) Loss of normal architecture of an Islet with loss of β-cells in diabetic control.
3) Regeneration and proliferation of β-cells and attainment of near normal architecture in glibenclamide group.
4) Near normal architecture of β-cells in Group IV.
5) Irregular Islet with extension into the exocrine portion in Group V.
6) Attainment of near normal architecture and hyper cellularity of Islets in Group VI treated at high dose

REFERENCES

(1) Andallu, B. and Radhika, B., (2000). Hypoglycemic, diuretic and hypocholesterolemic effect of winter cherry root. Indian J. Exp. Biol., 38:607-609 | (2)Ambati, S., Jyothi, V. and Jyothi, A.V., (2010). Pharmacological, pharmacognostic and phytochemical review of Embelia ribes. Int. J. Pharm. Technol., 2(4): 525-539 | (3)Bhandari, U. and Ansari. M.N., (2008). Antihyperglycaemic activity of aqueous extract of Embelia ribes burms in streptozotocin-induced diabetic rats. Indian J. Exp. Biol., 46: 607–613 | (4)Bhandari, U., Jain, N., Ansari, M.N. and Pillai, K.K., (2008). Beneficial effect of Embelia ribes ethanolic extract on blood pressure and glycosylated hemoglobin in streptozotocin-induceddiabetes in rats. Fitoterapia, 79: 351–355 | (5)Bhandari, U., Chaudhari, H.S., Bisnoi, A.N., Kumar, V., Khanna, G. and Javed, K., (2013). Anti-obesity effect of standardized ethanol extract of Embelia ribes in murine model of high fat diet-induced obesity. Pharmanutrition, 1: 50–57 | (6)Bopanna, K.N., Kannan, J., Sushma, G., Balaraman, R. and Rathod, S.P., (1997). Antidiabetic and antihyperlipidaemic effects of neem seed kernel powder on alloxan diabetic rabbits. Indian J. Pharmacol.,29: 162-167 | (7)Chopra, R.N., Nayar, S.L., and Chopra, I.C., (1999). Glossary of Indian medicinal plants. Edn.5th., New Delhi: National Institute of Science Communication., pp 106 | (8)Deb, L. and Dutta, A., (2006). Diabetes mellitus its possible pharmacological evaluation techniques and naturopathy. Int. J. Green Pharm., 1(1): 15–28 | (9)Gandhi, R.G., Stalin, A., Balakrishna, K., Ignacimuthu, S., Paulraj, M.G. and Vishal, R., (2013). Insulin sensitization via partial agonism of PPARγ and glucose uptake through translocation and activation of GLUT4 in PI3K/p-Akt signaling pathway by embelin in type 2 diabetic rats. Biochim. Biophys. Acta., 1830: 2243–2255 | (10)Gupta, O.P., Anand, K.K., Ali, M., Ghatak, R. and Atal, C.K., (1976). In vitro anthelmintic activity of disalts of embelin. Indian J. Exp. Biol., 14: 356–357 | (11)Javed, I. and Akhtar, M.S., (1990). Screening of Vernonia anthelmintica seed and Embelia ribes fruit mixed in equal parts against gastrointestinal nematodes. Pak. J. Pharm. Sci., 3: 69–74 | (12)Mhaskar, M., Joshi, S., Chavan, B., Joglekar, A., Barve, N. and Patwardhan, A., (2011). Status of Embelia ribes burm f. (Vidanga), an important medicinal species of commerce from northern Western Ghats of India, Curr.Sci., 100(4): 547-552 | (13) Naik, S.R., Niture, T.N., Ansari, A.A. and Shah, P.D., (2013). Anti-diabetic activity of embelin: Involvement of cellular inflammatory mediators, oxidative stress and other biomarkers. Phytomedicine,20: 797– 804 | (14)Pandey, A.K.and Ojha, V., (2011). Estimation of embelin in Embelia tsjeriam-cottam fruits by HPLC to standardise harvesting time, Indian J.Pharm. Sci., 73(1): 216- 219 | (15)Rani, A. S., Saritha, K., Nagamany, V. and Sulakshana, G., (2011). In vitro evaluation of antifungal activity of the seed extract of Embelia ribes, Indian J. Pharm. Sci., 73(1): 247-249 | (16)Tomlinson, K.C., Gardiner, S.M., Hebden, R,A. and Bennet, T., (1992). Functional consequences of streptozotocin induced diabetes with particular reference to cardio vascular system. Pharmacol. Rev., 44: 103 | (17)West, E., Simon, O.R. and Morrison, E.Y., (1996). Streptozotocin alters pancreatic beta-cell responsiveness to glucose within six hours of injection into rats. West. Indian Med. J., 45: 60-62