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EFFECT OF ANTIDIABETIC ACTIVITY OF *GYMNOSPORIA MONTANA* LEAVES ON DIABETIC RATS

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ABSTRACT

Gymnosporia montana (GM) belonging to the family Celastraceae commonly known as Bharatti and Vikalo. It is a shrub or tree growing wild in dry areas and is commonly found in Maharashtra, Gujarat and other part of India. It is widely used in treating sore, ulcer, gastro-intestinal disorders, toothache, and dysentery. The dried powder of leaf of *Gymnosporia montana* was subjected to the phytochemical screening for the presence of various phytoconstituents like alkaloids, flavonoids, saponins, tannins, anthraquinones, and carbohydrates, etc. Effectiveness of different extracts of leaf of *Gymnosporia montana* was evaluated (100 and 200 mg/kg; p.o.) against Streptozotocin (STZ) induced diabetes mellitus in rat for blood glucose level and blood parameters. Further, ethanolic extract of the plant was also evaluated (200 mg/kg; p.o.) for glycosylated haemoglobion study. The result of study show ethanol and aqueous extract powder of *Gymnosporia montana*, an popular herbal drugs, appears to be a safe alternative to reducing blood glucose.

KEYWORD: STZ, G*ymnosporia montana*, Blood glucose level, diabetic rats.

INTRODUCTION

Diabetes mellitus is a global burden as its incidence is considered to be high (4–5%) all over the world.

However, quest for the development of more effective antidiabetic agents is being pursued relentlessly.

Recently, herbal products have started gaining importance as complementary and alternative medicine to treat diabetic mellitus.[1] Diabetics have significant accelerated levels of oxidative stress and this contributes massively to most neurological, cardiovascular, retinal, renal diabetic complications.[2] Panoply of defenses against oxidative stress has evolved and operate at distinct levels. They are reduced generation of reactive oxygen species, enhancement of antioxidant enzymes like- Superoxide dismutase (SOD), catalase, glutathione peroxidase (GPX), and glutathione reductase (GSH) and repair system at the level of DNA. Hyperglycemia significantly diminishes glutathione level slowering defenses against oxidative stress. N-acetyl cysteine a precursor of GSH inhibited the development of functional and structural abnormalities of peripheral nerves in experimental diabetes.[3] Though, many herbal products have been described for the treatment of diabetic mellitus, very few of them have been explored scientifically so far. Biological activities of medicinal plants are closely related to their elemental composition.

Gymnosporia montana (Family:Celastraceae) also known as Maytenus senegalensisis a plant widely distributed throughout India. It is commonly known as 'Vikalo' in Gujrati,'Baikal'in Hindi or 'Bharati' in Marathi. In Indian floras, the genus Maytenus molina (family:Celastraceae) goes under the name of *Gymnosporia montana* (Wt. & Arn.) Benth. & Hook. F. Two hundred species have been reported of which about 15 are available in India.[4] Flora of British India mentions 16 species of *Gymnosporia montana*- G. acuminate, Hook. F., G. neglecta, Wall. Cat., G. salicifolia, Laws.,G.oblanceolata, Laws., G. puberula, Laws., G. fruticosa, ThwaitesEnum., G.ovata, Wall. Cat., G.rothina, W & A., G. regulosa, Laws.,G. heyneana, W&A., G. falconeri, Laws., G. rufa, Wall., G. royleana, Wall. Cat. G. wallichiana, Sprenz, Syst., G. emarginata, Roth. Nov. and G. montana, Roxb.[5] *Gymnosporia montana* (FIG.1) is a much branched, spinescent shrub or small tree, occurring throughout the arid, dry areas of India. Its systematic taxonomic position is as follows.[6]

Table No. 1: Taxonomic Classification:

Kingdom	**Plant**
Sub Division	Spermatophyta
Division	Angiospermae
Class	Dicotyledoneae
Sub Class	Polypetalae
Order	Celastrales
Family	Celastraceae
Genus	*Gymnosporia*(Wt. &Arn.) Benth& Hook. f.
Species	montana
Sub Division	Spermatophyta

Table No. 2: Vernacular names:[7]

Hindi	Baikal, Kngani, Tondarsaijhad
Ajmere	Kakra
Bengali	Vaichigachha
Marathi	Bharatti, Bharuli, Vekal, Vekar,
Tamil	Kattanji
Telegu	Dantausi, Danti, Gajasinni
Sanskrit	Bahuphala, Brahmapadapa
Oriya	Gourokasa
Punjabi	Dajkar, Kharai

Distribution[7]

Throughout the arid, dry areas of India. Punjab, Sind, W.Rajputana, Gujarat, Khandesh, W.Peninsula, Deccan, C.Provinces, Afghanistan, Arabia, Mediterranean, Tropical Africa, Malaya, Australia.

Ecology and propagation[8]

The plant grows at elevations from near sea level, on the coast on sand, at forest margins, hillsides and on sea cliffs, often on limestone. Long, hot summers are needed for production of flowers and fruits. It is an out breeding tree and shows great variability. Seeds can be sown under glass in autumn and semi-ripe cuttings of root with bottom heat in summer the plant grows in moderately fertile, moist but well-drained soil in full sun with midday shade. Flowers appear in October to January, fruiting during January - February and fruit ripens in March to April; develops new leaves from June to August.

Morphology

Leaf – Leaves are simple, alternate or clustered, found in the axils of spines, on the spines or on small branches; sub-sessile, glabrous and exhibit a vast degree of polymorphism in their shape. Leaves are 3-8 cm long and 1-3 cm broad, apex acute, mucronate or obtuse, margin entire in the lower half and renulate in the upper half.

Stem – Stems are purplish brown in colour, hard, straight, pointed and hard spines, which are modified branches with single node from which leaf originates.

Bark- Bark is thin with fine longitudinal wrinkles on the outer surface and creamy white inner surface.

Phytochemistry

Leaves-Several compounds viz. tingenone, 3-O-acetyloleanolic acid, hexacosane, hexacosanol, n-triacontanol, betulin, a-amyrone, a-amyrin, a-amyrin, a-sitosterol, celacinnine and kaempferolhave been isolated from the leaves of Gymnosporiamontana. Presence of Galactose as free sugar and seven free amino acids including arginine, glutamic acid, alanine, proline, a-aminobutyricacid, palmitic acid.

Stem-Iguesterin, pristimerin, tingenone, a-amyrin, a-sitosterol and maytenonic acid from the stem, sesquiterpene pyridine alkaloid Emarginatine B and maytansine are also present.

Root- Iguesterin, pristimerin, tingenone, a-amyrin, and a-sitosterol, dukidol and a–amyrin, epigallocatechin, Emarginatine, two other sesquiterpene pyridine alkaloids have also been isolated from this plant.

Properties and uses[6]

In several Ayurvedic literatures like Bhavprakash, Nighantu Adarsh, Shaligram Nighantu, Vanaspati Shrusti, Aryabhishek, Shankar Nighantu, Vanaspati Chandrodaya, the plant has been mentioned for various uses. It is claimed to be useful in jaundice, inflammation and rheumatic pain, corneal opacity, ulcers, gastrointestinal disorders, dysentery, toothache and also as a vermifuge. According to Shaligram Nighantu it is used in jaundice, inflammation and to cure blood disorders. Nighantu Adarsh mentions its use in kamla (jaundice). In Vanaspati Srusti the use of ripe fruit has been mentioned as blood purifier and anti inflammatory.

Leaf juice is used in pandu (anaemia) and used as an eye drop to cure corneal opacity. Bark is used to kill lice and

in other infection on the head. The use of leaf juice in eye diseases particularly in opacity of cornea, inflammation and burning sensation has been mentioned in Aryabhishek. In Vanaspati Chandradaya the use of root pulp in rheumatic pain while gum, along with other medicines, in cholera has been advocated. Kirtikar and Basu mention the fruit as appetizing and digestive and its use in jaundice and enlarged spleen. Ground seeds with turmeric are recommended to be rubbed all over the body to prevent rheumatic pain from exposure to damp winds.

The external application of dry powdered leaves with a little mustered oil has shown encouraging result in rickets. In Saurashtra region of Gujarat, India, the leaf juice is well known for curing jaundice. Extract of leaves mixed with cow milk is taken in the morning for 3 days by the local people of Bhadra (Karnataka, India) for curing jaundice. The root bark is reported to be useful in dysentery.

MATERIALS AND METHODS

Plant material-Fresh leaves of *Gymnosporia montana* were collected and identified (Voucher specimen no.16563 B stored in herbarium of Bhavbhuti Mahavidyalaya Amgaon). The leaves were dried and cut into small pieces, the pieces were mechanically crushed. 4 kg of crushed leaves were continuously extracted with Pet. Ether (PEE), ethanol (EE) and distilled water (WTE) using soxlet.[9] The extract was filtered and concentrated in rotatory evaporator at 35–40 °C under reduced pressure to obtain a semisolid material, which was then lyophilized to get a powder.

Fig. 1: Gymnosporia Montana.

Experimental animals

S.D rats of approximately same age group, having body weight 160-200 g were used in the experiment. Animals were kept in our animal house at an ambient temperature of 27±3 °C and 50±5% relative humidity with a 12 h each of dark and light cycle. Animals were fed with pellet diet and distilled water. The study was approved by the P.Wadhwani College of Pharmacy Institutional animal Ethical Committee (IAEC) wide no.650/PO/Re/S/2002/CPCSEA/2016/12.

Induction of diabetes in rats

Diabetes was induced by single intraperitonial injection of freshly prepared solution of STZ at the dose of 50mg/kg in 0.1Mcitrate buffer (pH 4.5) to the rats fasted overnight.[10] After 3 days of STZ induction, FBG was checked and animals with abnormal FBG(>110 mg/dl) were treated as diabetic rats.

Estimation of BGL and detection of blood parameter

Blood glucose level was estimated by glucometer (Accucheck). Blood parameters were detected by using Ambica diagnostic AD-100 Biochemistry Analyzer Screening of the extract for hypoglycemic activity was done with a range of variable doses (100, 200 Mg/kg) in normal healthy rats by conducting fasting blood glucose (FBG) and glucose tolerance test studies.

Assessment of antidiabetic potential in diabetic rats

The antidiabetic effects of different extract in diabetic rats were assessed. The rats of diabetic models were divided into nine groups of six rats each. Group I is control, received vehicle (distilled water only), and Group II is diabetic control, Group III (Standard) treated with glimepiride. Whereas variable doses of 100 and 200 mg/kg of leaves extract was given orally to group IV, V, VI, VII, VIII and IX respectively. Blood glucose levels were checked in day 1,7,14 and 21. The results were compared with all groups of rats, treated with 0.8 mg/kg of Glimeperide, a reference drug.

Experimental design Protocol for Antidiabetic Activity-

Group I	Normal with daily dose of 0.5 ml of 0.5 % Tween 80 (vehicle) p.o
Group II	Diabetic control with single dose of Streptozotocine (50 mg/kg ip)
Group III	Diabetic rats with daily dose of 0.8 mg/kg Glimepiride in 0.5% tween 80 p.o.
Group IV	Diabetic rats with daily dose of 100 mg/kg PEE in 0.5% tween 80 p.o.
Group V	Diabetic rats with daily dose of 100 mg/kg EE in 0.5% tween 80 p.o.
Group VI	Diabetic rats with daily dose of 100 mg/kg WTE in 0.5% tween 80 p.o.
Group VII	Diabetic rats with daily dose of 200 mg/kg PEE in 0.5% tween 80 p.o.
Group VIII	Diabetic rats with daily dose of 200 mg/kg EE in 0.5% tween 80 p.o.
Group IX	Diabetic rats with daily dose of 200 mg/kg WTE in 0.5% tween 80 p.o

Table 3: % yield of different extract of Gymnosporia montana leaf.

Type of extract	Petroleum ether leaf extract	Ethanol leaf extract	Aqueous leaf extract
Color of extract	Dark greenish brown	Brownish green	Dark brown
All. 26 % yield from extract	4.6 %	12.5 %	7.9 %

Table 4: Quality control parameters of *Gymnosporia montana* leaf extract parameters

PARAMETERS	% W/W LEAF
Total ash	13.1±0.56
Acid insoluble ash	2.38±0.76
Water insoluble ash	3.15±0.66
Water soluble extractive values	13.9±0.47
Alcohol soluble extractive values	10.1±0.53
Petroleum ether extractive values	6.98±0.46
Foreign matter	0.7 ±0.09
Moisture content	5.2 ±0.15
Microbial contamination	0.2

Statistical analysis

Statistical analysis was performed using Data analysed by Two-way ANOVA followed by Bonferroni post tests using statistics. The significance of difference between and within various groups was determined.

Values expressed as mean ± S.D., n=6
$^{\#}P < 0.05$ when compared with vehicle treated group.
$^{*}P < 0.05$ when compared with diabetic control group.

RESULTS

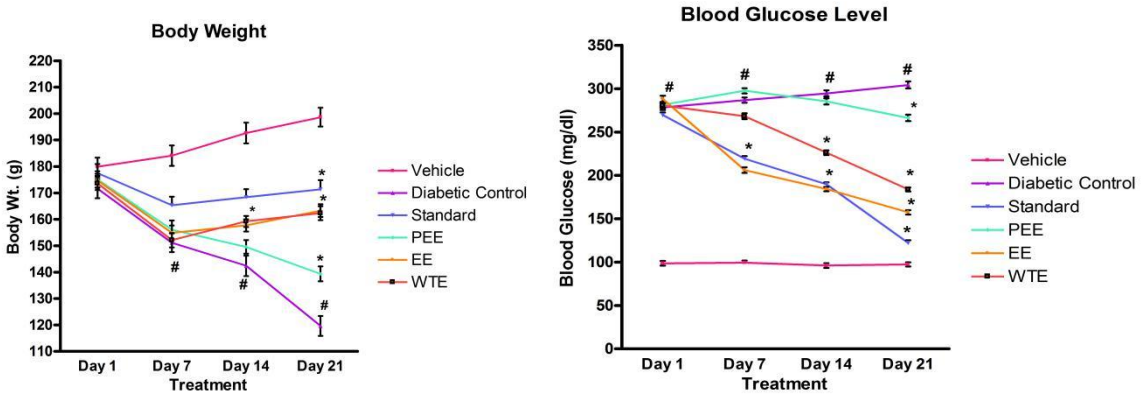

Fig. 1: The effect of three-week treatment with various extracts of *Gymnosporia montana* (100mg/kg) on body weight and Blood glucose level of SD rats.

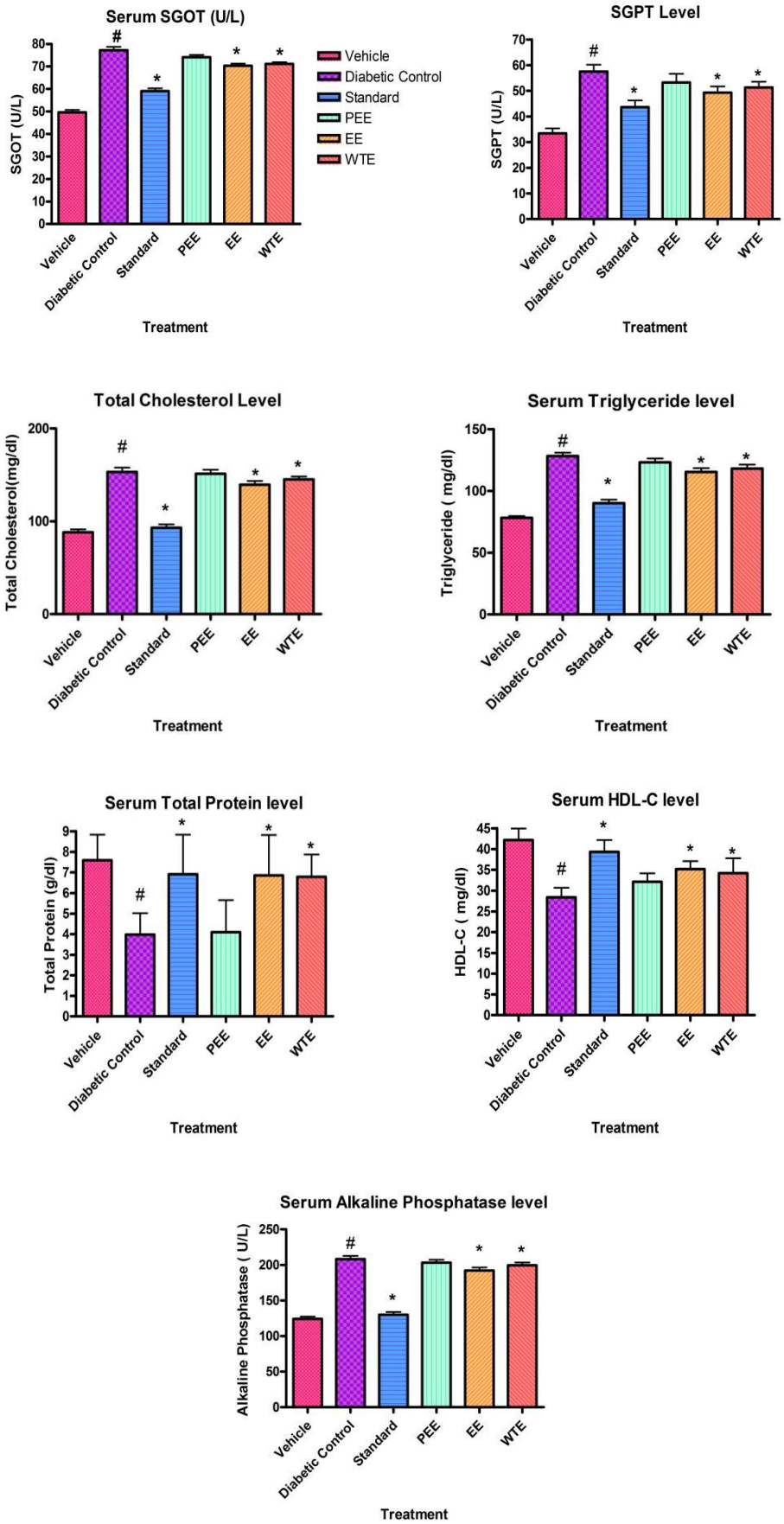

Fig. 2: Effect of *Gymnosporia montana*(100mg/kg) leaves extracts on different blood parameters of SD rats on day 21.

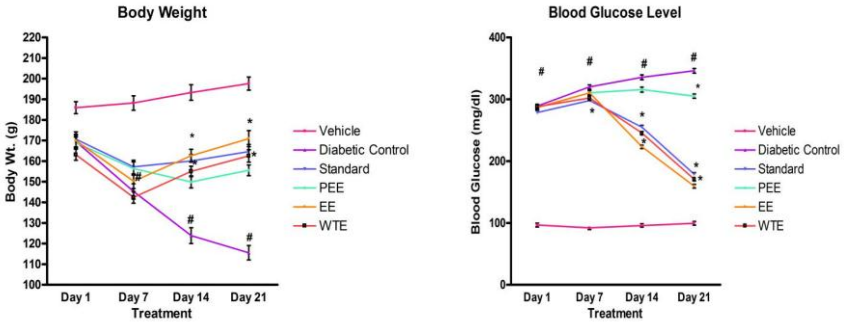

Fig. 3: The effect of three-week treatment with various extracts of *Gymnosporia montana* (200 mg/kg) on body weight and blood glucose level of SD rats.

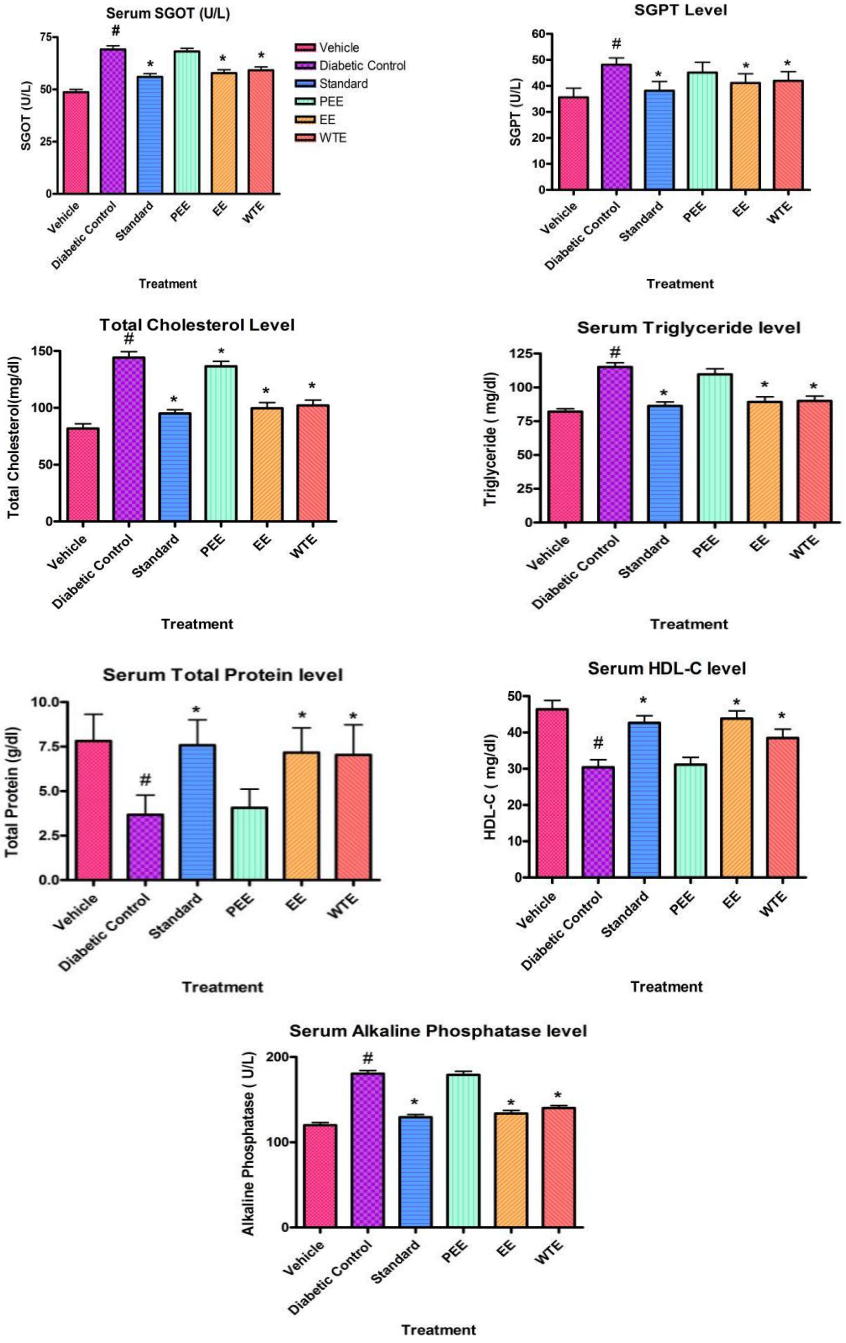

Fig. 4: Effect of *Gymnosporia montana*(200mg/kg) leaves extracts on different blood parameters of SD rats on day 21.

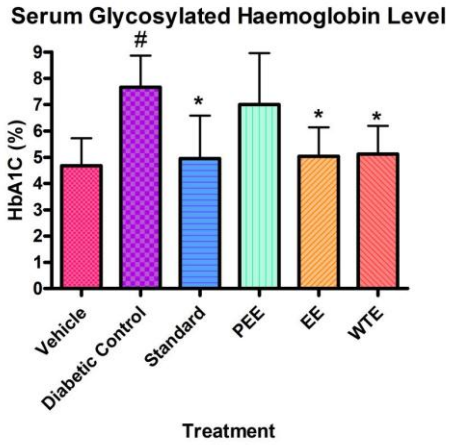

Fig. 5: Effect of oral administration of *Gymnosporia montana* leaves extract (200 mg/kg)on Glycosylated Haemoglobin of SD rats on day 21.

RESULTS AND DISCUSSION

Diabetes Mellitus is a metabolic disorder characterized by a loss of glucose homeostatis with the disturbance of carbohydrates, fat, protein metabolism resulting from defects in insulin.[13] In our study, diabetes was induced in rats by single intraperitoneal injection of STZ (50mg/kg b.w)[14] and the, antidiabetic activity of *Gymnosporia montana* leaves was determined. Treatment of Diabetes mellitus with oral hypoglycemic agents like sulphonylurea and biguanide is associated with severe adverse effects.[15] Therefore, herbal drugs are gaining importance in the treatment of various diseases. The administration of STZ to the normal rats results in the destruction of beta cells of Islets of Langerhans and malfunctioning of the pancreas. This results in the diabetic condition leading to the increase in the blood glucose levels and decreased body weight in the untreated diabetic rats. Due to the action of STZ, the beta cells undergo destruction of necrosis.[16] The present study reports the effect of *Gymnosporia montana* as a antihyperglycemic agent thus scientifically validating the traditional claim. Pet. ether extract(PEE) of *Gymnosporia montana* leaves doesn't show any effect, aqueous extract (WTE) shows mild improvement in blood glucose level but the ethanolic extract at the dose of 200 mg/kg had shown a significant antihyperglycemic effect in diabetic rats at third week after treatment. The probable mechanism of action of hypoglycemic activity extract may be due effect against free radicals which prevent pancreas damage it result into improvement in blood sugar level, however it has already been reported that this antihyperglycemic action might be due to modulation of insulin secretion and/or insulin action or could be related to the interference on absorption of dietary carbohydrates as well as disaccharides in small intestine leading to the suppression of meal induced increase of plasma glucose.

In the present study, streptozotocin produced significant increase in plasma glucose level by 270-305mg/dl by selectively destroying the pancreatic insulin secreting β cells causing diabetes close to type 2 diabetes of humans. After 21 days treatment, 100 mg and 200 mg/kg, ethanol extract of *Gymnosporia montana* leaves had reduced both the fasting blood glucose levels in streptozotocin induced diabetic rats.

In this study, diabetes control rats exhibited significantly elevated SGOT, SGPT, cholesterol and triglyceride levels as compared to normal control rats. Treatment with ethanolic and aqueous extract significantly reduced SGOT, SGPT, cholesterol and triglyceride levels in dose dependent manner. It also improves total protein and serum HDL cholesterol. Maintenance of serum lipid profiles recommended the effectiveness of the extract against experimental type 2 diabetic rats.

CONCLUSION

In conclusion, our study adds credence to the traditional use of *Gymnosporia montana* leaves by the vidarbha to treat diabetes.

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