Section A-Research paper
ISSN 2063-5346

Phytoconstituent and Pharmacological screening for anti-diabetic activity of *Tephrosia villosa*

[1]Sheik Nasar I, [2]Azra Aisha, [3]M. Naveen Kumar[*], [4]Hemant P. Suryawanshi, [5]Rekha Bisht, [6]Sushila Gupta, [7]Yashvardini S. , [8]Mohammad Khalid

[1]Associate Professor, East Point College Of Pharmacy, East Point Group Of Institutions, Jnana Prabha ,East Point Campus,Virgo Nagar ,Bidarahalli, Bengaluru, Karnataka.560049

[2]Associate Professor, Era College of Pharmacy, Era University, Sarfarazganj, Lucknow, Uttar Pradesh. 226003

[3]Associate professor, Vaageswari College Of Pharmacy, Beside LMD Police Station, Ramakrishna Colony, Thimmapur, Karimnagar, Telangana, India. 505547

[4]Professor, PG College of Pharmaceutical Science and Research, Chaupale, Nandurbar, Maharashtra. 425411

[5]Professor, Indore Institute of Pharmacy, Rau-Pithampur road, Indore, Madhya Pradesh. 453331

[6]Research Scholar, Adarsh Vijendra Institute of Pharmaceutical sciences, Shobhit University Gangoh, Saharanpur, Uttar Pradesh.

[7]Student, Sri Ramakrishna Institute of Paramedical Sciences - College of Pharmacy, 395, Sarojini Naidu Rd, Siddhapudur, Balasundaram Layout, B.K.R Nagar, New Siddhapudur, Coimbatore, Tamilnadu. 641044

Associate Professor, Krishna Pharmacy College, Moradabad Road, Bijnor, Uttar Pradesh, India. 246701

Corresponding Author

[3]M. Naveen Kumar*

[3]Associate professor, Vaageswari College Of Pharmacy, Beside LMD Police Station, Ramakrishna Colony, Thimmapur, Karimnagar, Telangana, India. 505547

doi: 10.48047/ecb/2023.12.si4.1426

Abstract

The *Tephrosia villosa* (L.) Pers herb extract were prepared by successive soxhlation i.e. extracting dried powder with the solvents of increasing order of polarity, and It was qualitatively observed that 70% ethanolic extract contain higher concentration of polyphenol, flavonoid, alkaloid, protein and tannin components andquantative studies of The total phenolic content of 70% EETVH was 1.08 mg/G expressed as equivalent to catechol. Similarly flavonoid content was found to be 3.21 mg/G expressed as equivalent to quercetin and total tannin content found to be 5.10 mg/G expressed as equivalent to tannic acid.

Anti-diabetic property of the 70 % EETVH was studied in *In-vitro* and *In-vivo* models, . In-vitro non enzymatic glycosylation of haemoglobin method 70% EETVH significantely inhibited the hemoglobin glycosylation which is indicated by the presence of increasing concentration of hemoglobin, the plant extract exhibited higher inhibition indicating plant extract decreases the formation of glucose hemoglobin complex. *In-vivo* anti-diabetic activity of 70% EETVH is

carried out in experimentally induced alloxan –diabetes in albino rats and to compare activity the lower dos($1/10^{th}$ cutoff value)and higher dose ($1/5^{th}$) are selected from the toxicity studies according to OECD guidline no-423. It implies that 70% EETVH significantly reduces the elevated blood glucose .

Keywords: *Tephrosia villosa* (L.), invitro diabetic activity, invivo diabetic activity, alloxan

INTRODUCTION

Diabetes mellitus is a common metabolic disorder characterized by chronic hyperglycaemia, with disturbances of carbohydrate, fat and protein metabolism resulting defects in insulin secretion, action or both[1] it is one of most serious endocrine metabolic disorder has caused significant mortality and morbidity due to micro vascular (Retinopathy ,Nephropathy)and macro vascular (Heart attack, stroke) complications[2] blood glucose is essential to life as a regulator of Homoeostasis, glucose is the obligate metabolic fuel for the brain under the physiologic condition, glucose in plasma either comes from dietary source or by the result of the breakdown of glycogen in liver or formation in the liver and kidney from other carbon compounds such as Lactate, pyruvate aminoacids and glycerol.[3] Insulin is very essential for glucose uptake into the cell, it is an hormone secreted by the beta cells of pancreas if pancreas does not produce enough insulin glucose get into the blood cells and stays in the blood this results in hyperglycaemia.[4]

DM is classified into two major subtypes: type I (insulin dependent diabetes mellitus (IDDM) and type II (non-insulin dependent diabetes mellitus (NIDDM).and Gestational diabetes. IDDM or juvenile onset diabetes results from a cellular mediated autoimmune destruction of the β-cells of the pancreas. However, NIDDM or adult-onset diabetes results from the development of insulin resistance and the affected individuals usually have insulin deficiency. Patients suffering from type I are therefore totally dependent on exogenous source of insulin while patients suffering from Type II diabetes can be treated with dietary changes exercise and medication. Type II diabetes is the more common form of diabetes constituting 90% of the diabetic population[5].

International Diabetic Federation reported that The global diabetes prevalence in 2019 is estimated to be 9.3% (463 million people), rising to 10.2% (578 million) by 2030 and 10.9% (700 million) by 2045. The prevalence is higher in urban (10.8%) than rural (7.2%) areas, and in high-income (10.4%) than low-income countries (4.0%). One in two (50.1%) people living with diabetes do not know that they have diabetes. The global prevalence of impaired glucose tolerance is estimated to be 7.5% (374 million) in 2019 and projected to reach 8.0% (454 million) by 2030 and 8.6% (548 million) by 2045. high prevalence of diabetes has important social, financial and developmental implication especially In low and middle income countries,[6] The world Health organization projected that diabetes will be the 7^{th} leading cause of death.

Plant description:
Name of the plant : *Tephrosia villosa* (L.) Pers

Synonym [a] :
Cracca incana Roxb.
Cracca villosa L.
Cracca villosa L. var. *incana* (Roxb.) Hiern
Galega hirta Buch.-Ham.
Galega incana Roxb.
Tephrosia ehrenbergiana Schweinf.
Tephrosia hirta (Buch.-Ham.) Benth.
Tephrosia incana (Roxb.) Wight & Arn.
Tephrosia incana (Roxb.) Wight
Tephrosia villosa (L.) Pers. var. *argentea* Thwaites
Tephrosia villosa (L.) Pers. var. *incana* (Roxb.) Baker
Taxonomy
Kingdom : Plantae
Division: Magnoliophyta
Class : Magnoliopsida
Order: Fabales
Tribe : Millettieae
Family: Leguminosae (Fabaceae)- Papilionoideae(L.) Persoon
Genus: *Tephrosia*
Species: *villosa Pers*.

The plant is known by various names in different languages as under. [7]

English :	Shaggy wild indigo
Sanskrith :	**S**harpunkha
Hindi :	Salunak.
Kannada:	Niligida
Tamil :	Kottukolingi
Oreia:	Kulthia, Piderkalata

Plant Form : Bushy **Herb,** shrub
Flower: In November and fruit in February in india
Distribution: *Tephrosia villosa* has a large distribution found in southern and eastern Africa, the Arabian Peninsula and across southern Asia.
Botanical Discription: Sharpunkha" an important ayurvedic drug has been in use for a long time two kinds of sharpunkha the shevet (white)and Raktha (Red) are described in some of the ayruvedic texts like Vaghbhatas "Astang Hridayam"and in Nighantus[8]. shevet sharpanakha is

Tephrosis villosa due to its persistently villous like white part and this view receives the support also from "Shivadatta Nighantu"which mentions "Sharpunkheti vikhya shreepuspha kavachid bhavet" Genus Tephrosia Pers is a large seasonal pantropic genus of about 400species belongs to family Fabaceae, in india genus *Tephrosia* Pers is represented by 29 taxa including 27 species 1 variety and 1 sub species out of which 7 taxa are endemic to india.in india maximum taxa of Tephrosia are recorded in presidency of Madras. (Gamble,1957)[9]

The name is from greek word Tephros meaning ash-coloured, reffering to the grayish tint given to the leaves and pod by their dense hairs.

Genus *Tephrosia* Pers is characterized by herbs or shrubs

leaves stipulate,pinnate,imparipinnate ,rarely simple

Flowers generally in terminals or leaf opposed racemes.calyx lobes subequal,

petals clawed, standard sub orbicular, stamens diadelphous,

pods usually linear, flattened,many seeded,continuous or scarcety septate(Hooker 1961) some of the species are cultivated as cover crops,green manures,fish poision and ornamental.

Dignostic characters of Tephrosia are

1. Leaves cut into forks (Horn like structure).
2. Pods flat, not joined many seeded.
3. Standard petal obtuse.
4. Sepals subequal, calyx lobes connate.
5. Anthers muticous, basifixed

Tephrosia villosa is an annual or perennial bushy herb, 0.3-1.3 m tall. Stem white tomentose. Leaves imparipinnately compound with 7-19 leaflets, up to 10 cm long;stipules 2-5 mm long; leaflets obovate to elliptical, up to 21 mm x 9 mm,hairy on both sides, each side with 4-8 pairs of distinct veins. Stipulestomentose, caducous and lanceolate. Flowers in a terminal or upper axillary pseudo raceme 8-22 cm long; pedicel with densely matted hairs, 2-4 mm long; calyx densely matted hairy, tube about 2 mm long, lobes long-acuminate, to 9 mm long;standard transversely elliptical to broadly ovate, up to 7 mm x 10 mm,dorsally with dense brown hairs. Style glabrous, up to 3-5 mm long, bent sharply upward at base, twisted, penicillate. Pod strongly curved, up to 4 cm x 6 mm, densely silvery or brown tomentose,hairs to 2 mm long, 4-10-seeded. Seed 12-16, rectangular, black, smooth, with short hard excrescences, upto 4.5 mm x 2.5-2.75 mm. Flower in November and fruit in February in India. [14] The specific name 'villosa' means covered in white soft hair in Greek

ISSN 2063-5346

Photographs of **Tephrosia villosa Pod**

Photographs of **Tephrosia villosa Herb**

Tephrosia villosa (per) has been found to be having
Antimicrobial and Brine shrimp activity [10-11]
Antidiabetic activity [12]
Potential bio insecticide [13]
Anthelmintic activity [14]
Antioxidant activity [15]
Green corrosion inhibiter activity [16]

EXPERIMENTAL WORK

I. Preparation of extract

The *Tephrosia villosa* (L.) Pers herb extract will be prepared by successive soxhlation i.e. extracting dried powder with the solvents of increasing order of polarity i.e. Pet. ether (60-80°), Chloroform (59.5-61.5°), 70% Ethanol (64.5-65.5°) . Extracts will be concentrated under reduced pressure. and stored in airtight container in refrigerator below 10 ^{o}C.

II. Preliminary phytochemical screening

III. Quantitative determination by Spectrophotometry

- Estimation of Total Phenolic Content
- Estimation of Total Tannin Content
- Estimation of Total Flavonoid Content

IV. Isolation of phytoconstituents

V.Structural elucidation by spectroscopic methods

VI. In vitro anti diabetic activity

1.Non-enzymatic glycosylation of haemoglobin method

2.Glucose uptake in Yeast cells

3.Alpha- Amylase inhibition assay

4. Glucose Adsorption Assay.

VII.Determination of acute toxicity

VIII. Screening of Alloxan induced anti diabetic activity and analysis of fallowing parameters

- Body weight of an animal.
- Biochemical parameters such as

a) Fasting blood glucose
b) Serum total cholesterol
c) Serum creatinine
d) Serum urea
e) Serum protein
f) Serum triglyceride
g) Serum HDL
h) Serum LDL
i) Hepatic glycogen estimation.

- Histopathological studies

II. Preliminary phytochemical screening

The obtained extract will be subjected to preliminary phytochemical screening following the standard procedures described in the practical Pharmacognosy by C.K. Kokate [17] and R.K. Khandelwal [18] results are summarized in table no……..

1. Detection of carbohydrates

2. Detection of proteins and amino acids

3. Detection of alkaloids

4. Detection of flavonoids

5. Detection of tannins

6. Detection of diterpenes.

7.Detection of steroids and triterpenoids

8. Detection of saponins

9. Detection of cardiac glycoside

- **Keller-Kiliani's** test: A portion of dry extract is treated with 1mL of FeCl3 reagent (1 volume of 5% FeCl3 and 99 volume of glacial acetic acid). To this solution a few drops of concentrated H2SO4 is added. The presence of greenish blue color within a few minutes indicates the presence of desoxy sugar of cardiac glycosides.
- **Baljet's test**: To 1mL of the extract, 1mL of sodium picrate solution is added and the appearance of yellow to orange color reveals the presence of carbenolide

IV. **Isolation of phytoconstituents and Structural elucidation by spectroscopic methods**

GC-MS characterization of plants extracts

The GC-MS analyses were carried out in Shimadzu GC-MS QP2010 gas chromatograph fitted with DB1 capillary column ,carrier gas Helium with flow rate of 0.7mL\min, column oven temperature 70 ^{o}c,5 min 180 ^{o}c,5 min in 260^{o}c and finally 5 min in 280^{o}c, volume injected 1 microlt in n-hexane (2%) split ratio 3:0 the MS operating parameters were as fallows ionization potential 70ev; ion source temperature 200^{o}c, solvent dealy 6.0min, scan speed 2000amu/s, the concentrated extract injected into GC-MS instrument the sample was volatilized at the injection port and eluted through a capillary column under increasing temperature

^{13}C and ^{1}H- Nuclear Magnetic Resonance (NMR)

The purified plant extract was dissolved in Chloroform-D and subjected to ^{13}C and ^{1}HNMRspectroscopic studies 500MHz (Bruker advance II). ^{13}C-spectra gives the information about various carbon functional groups while ^{1}H- NMR indicates the total number of protons associated with several groups.

In-vitro Antidiabetic activity

1.Non-enzymatic glycosylation of haemoglobin method [18]

In-vitro *Antidiabetic* activity of *Tephrosia villosa* (L.) Pers herb extract. Was investigated by estimating degree of non-enzymatic haemoglobin glycosylation measured colorimetrically at 520nm described by Chandrashekhar *et al*. Glucose (2%), haemoglobin (0.06%) and Gentamycin (0.02%) solutions were prepared in phosphate buffer 0.01 M, pH 7.4. 1 ml each of above solution was mixed.70% alcoholic extract of *Tephrosia villosa* (L.) Pers herb. was weighed and dissolved in DMSO to obtain stock solution and then 1-5 µg/ml solutions were prepared. 1 ml of each concentration was added to above mixture. Mixture was incubated in dark at room temperature for 72hrs. The degree of glycosylation of haemoglobin was measured colorimetrically at 520nm. Alpha-Tocopherol (Trolax) was used as a standard drug for assay.

% inhibitionwas calculated as-% inhibition= A_S---$A_{C/}$00As #

Ac is Absorbance of Control

As is Absorbance of Sample

Statistical Analysis- All determinations were carried out in triplicates and data were expressed as mean +_ sem , all analysis were carried out using graph pad prisim 6 software significant at $p \leq 0.05$.

Pharmacological activities.

TOXICITY STUDY

Acute oral toxicity study (OECD 423) [19]

Toxicity studies are carried out according to OECD guidelines 423 was
followed. It is a stepwise procedure with three animals of a single sex per step. Depending on the mortality and/or, 50, 300, 2000mg/kg body weight) and the results allow a substance to be ranked by morbidity of the animals a few steps may be necessary to judge the toxicity of the test substance. This procedure has advantage over other methods because of minimal usage of animals while allowing for acceptable data.

The method uses defined doses (5and classified according to the globally harmonized system. The starting dose for ethanolic extract was
2000mg/kg bodyweight (p.o). The dose was administered to the rats which were fasted overnight with water ad libitum and observed for signs of toxicity. The same dose was once again tried with another three rats and were observed for 72 hours for symptoms like change in skin colour,salivation, diarrhea, sleep, tremors, convulsions and also respiratory,autonomic and CNS effects.

Alloxan induced anti diabetic activity.[20-22]

Induction of diabetes

Alloxan (2,4,5,6-tetraoxypyrimidine; 2,4,5,6- pyrimidinetetrone) is an oxygenated pyrimidine derivative and was originally isolated in 1818 by Brugnatelli and got its name in 1838 by Friedrich Wöhler and Justus von Liebig (17). Alloxan is a toxic glucose analogue, which selectively destroys insulin-producing cells in the pancreas when administered to rodents and many other animal species. This causes an insulin-dependent diabetes mellitus (called "Alloxan Diabetes") in these animals, with characteristics similar to type 1 diabetes in humans (Lenzen, 2008). Alloxan monohydrate was obtained from S.D. Fine, Mumbai and all the other chemicals used were of analytical grade and were acquired from commercial sources.

The various groups used in experiment:

Group I - Served as normal control and did not receive any treatment.

Group II -Served as diabetic control and received alloxan monohydrate and vehicle Group III - Alloxan + Glibenclamide (10 mg/kg p.o.) and served as standard.

Group IV - Alloxan monohydrate + 70% EET VH(250 mg/kg, p.o.)

Group V - Alloxan monohydrate + 70% EETVH (500 mg/kg, p.o.)

Care of Diabetic Animals:

Since diabetic animals drink large amount of fluid and produce large volume of urine, the bedding is changed frequently, usually every day and in some circumstances, more than once per day. Diabetic rats should have sufficient food and water.

Collection blood and serum samples:

The blood was drawn from the retro orbital plexus of the rats (fasted for 14 h) under light ether anesthesia on different occasion, i.e., 0, 4^{th}, 7^{th} and 10^{th} day. The blood samples were allowed to clot

for 30min at room temperature and then they were centrifuged at 3000 rpm for 10min. The resulting upper serum layer was collected in properly labeled, clean and dry micro-centrifuge tubes. The serum samples were stored at - 40^{o} C and analyzed either immediately or within two weeks.

- The parameters studied were as follows:
- Body weight of an animal
- Biochemical parameters such as

j) Fasting blood glucose
k) Serum total cholesterol
l) Serum creatinine
m) Serum urea
n) Serum protein
o) Serum triglyceride
p) Serum HDL
q) Serum LDL
r) Hepatic glycogen estimation.

- Histopathological studies.

Table No.1 Phytochemical screening of *Tephrosia villosa* (L.)

Sl. No.	Solvent	Colour and Consistency	Percentage yield
1	Pet. Ether	Greenish black sticky	1.92%
2	Chloroform	Brownish black and sticky	3.95%
3	70% Ethanol	Brownish black and non sticky	18.31%
Types of Phytochemical constituents	Petroleum ether Extract	Chloroform Extract	70/ Alcoholic Extract
Alkaloids	+	+	+++
Carbohydrates	-	–	+++
Flavonoids	–	–	+++
Glycosides	-	-	+

Tannins and Poly phenol	–	–	+++
Protein	-	-	+
Steroids	-	-	++
Saponin	-	_	+

Table No. 2 .:*In-vitro*Non-enzymatic glycosylation of haemoglobin method

S. No	Conc.n (µg/ml)	Blank	STD		EETVH	
			Abs	% inhibition	Abs	% Inhibition
1	100		0.104±0.002	69.23	0.109±0.002	70.64
2	200	0.032+0.001	0.120±0.002	73.33	0.125±0.001	74.40
3	300		0.127±0.002	74.80	0.137±0.001	76.64
4	400		0.135±0.003	76.29	0.150±0.001	78.66
5	500		0.141±0.001	77.30	0.159±0.002	79.87

STD- Standard, , Abs- Absorbance, % inh- % inhibition, Conc.n- Concentration EETVH-70% Alcoholic extract, Values are expressed as mean ± SEM.(N=3)

Figure No.1

Fig No..... Non-enzymatic glycosylation of haemoglobin

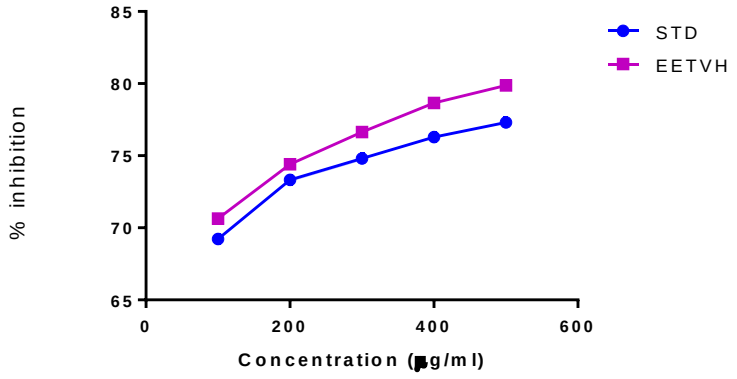


Table No. 3: Effect of 70% EETVH on body weight in alloxan induced diabetic rat

Groups	**Dose (mg/kg)**	**Intial**	***4th day***	***7th day***	***10th day***
		Mean ±SEM	**Mean ±SEM**	**Mean ±SEM**	**Mean ±SEM**
Control	**Vehicle**	**199.4** ±2.017	202.0 ±1.713	**203.0** ±1.183	209.5 ±2.741
Diabetic control	**Alloxan(120mg/Kg)**	201.5 ±3.423	181.7 ±2.076	156.8±1.222	151.8 ±2.480
standard	**Alloxan(120mg/Kg) +Glibenclamide (10mg/KG)**	199.0 ±1.932	198.3 ±1.978	192.2±2.372***	189.0 ±1.520***
Lower dose	**Alloxan(120mg/Kg)+70%EETVH(250mg/kg)**	202.3 ±2.512	194.3 ±2.011	183.3±1.783**	181.7 ±2.241**
Higher dose	**Alloxan(120mg/Kg)+70%EETVH(500mg/kg)**	199.7±2.155	197.7 ±2.155	188.8 ±1.783**	183.8 ±1.441***

Figure 2

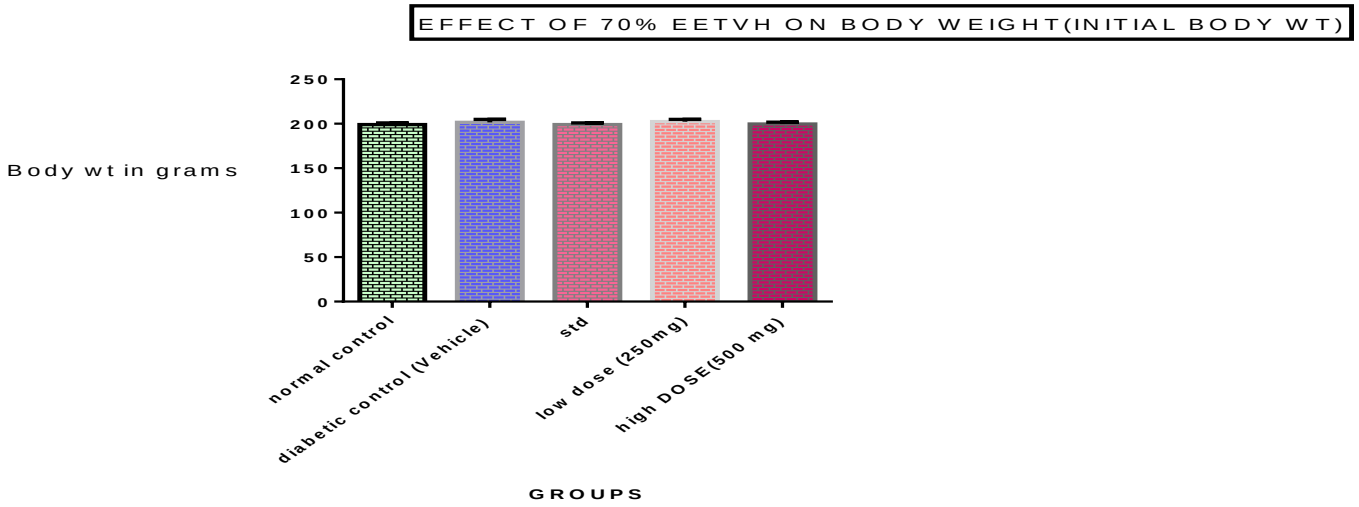


Figure 3

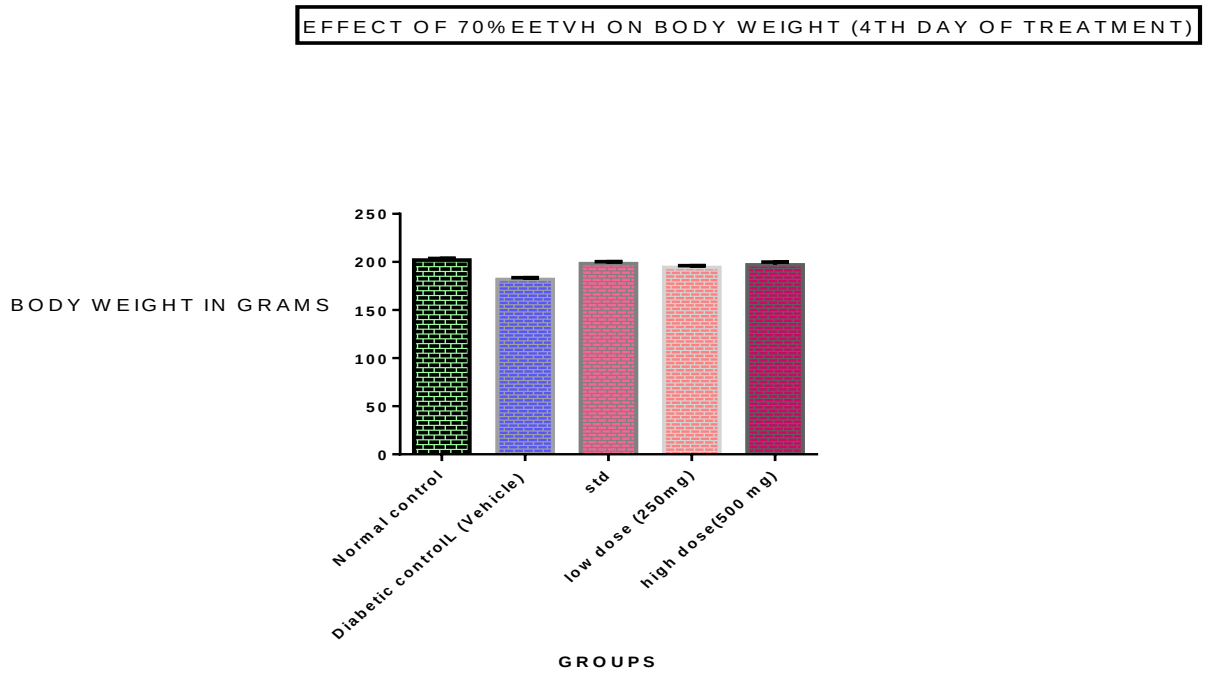


Figure 4

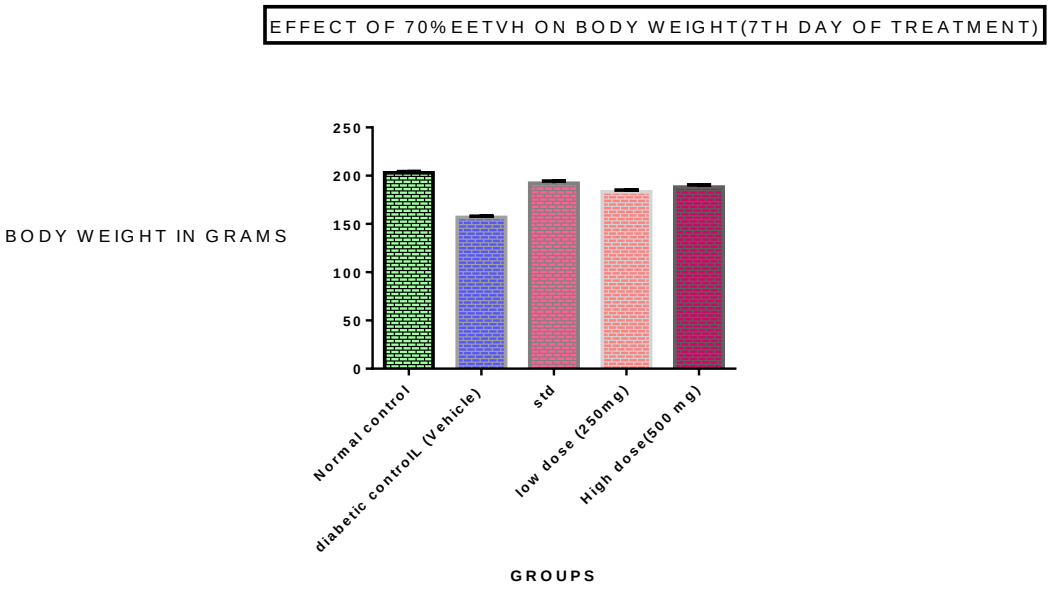
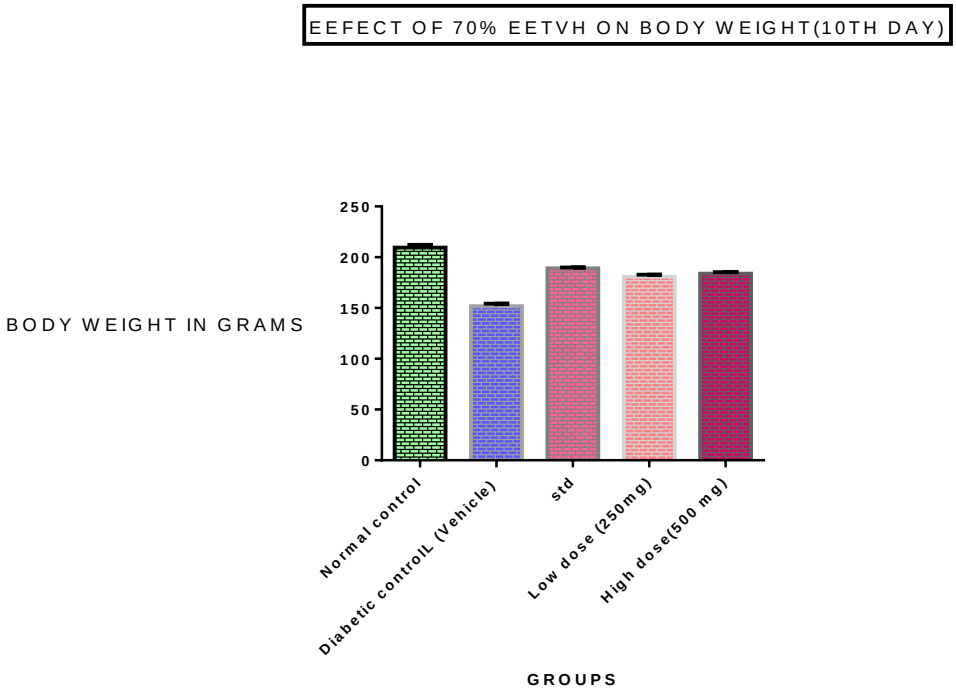


Figure 5



CONCLUSION

Diabetes mellitus. a major disorder in the world leading to massive economic losses. Diabetes is a chronic disorder of carbohydrate, fat and protein metabolism characterized by increased fasting and fed blood sugar levels in the human body. World health organization predicted that the

developing countries will face major burden due to Diabetes. Studies conducted in India, highlighted that the prevalence of diabetes is high and also the rapid increase in the urban population.

Prolonged medications required for the treatment of diabetes mellitus, considering the limitations and side effect in the synthetic drugs, natural products derived from medicinal plants are being looked for the treatment of diabetes. Considering low toxic, ready availability and cost effective, usage of natural products for the treatment of diabetes mellitus has recommended and encouraged by WHO (World Health Organization) worldwide.

Plants have always been good source of drugs the ethnobatonical information reports many plants that may posses anti- diabetic potential *Tephrosia villosa* has a large distribution found in southern and eastern Africa, the Arabian Peninsula, across southern Asia and in south India and phytoconstituent anyless shown that the presence of polyphenols,Tannins,Alkaloids and Terpenoids in 70% alcoholic extract so it is selected.

Comp-1 was isolated from the plant extract and it was characterized and identified by structural elucidation In-vitro anti-diabetic studies shows that the plant extract posses good anti-diabetic property and its is conformed in the In-vivo anti-diabetic studies.

From the results and discussion it is concluded that the plant possess significant anti-diabetic property However, further research is required to elucidate the specific mode of action.

References

1.S.R.Paul, R.C.Gupta (1988) Pharmacobotonical studies on "Shevet Sharpunkha" – a comparative diagnostic account of *Tephrosia villosa per* and *T. Purpurea(linn).pers.*ancient science of life,vol no.VII Nos.3&4,January&April ,pages207-21.

2..Flora of the Presidency of Madras,J.S.Gamble vol.I published under the Authority of the Secretary of State for India in council London , Adlaed & Son,limited 21,Haet street, W.C. https://doi.org/10.5962/bhl.title.21628

3. Orwa, C.; Mutua, A.; Kindt, R.; Jamnadass, R.; Anthony, S., (2009). Agroforestree Database: a tree reference and selection guide version 4.0. World Agroforestry Centre, Kenya http://www.worldagroforestry.org/resources/databases/agroforestree

4.Soumith K Behra,Anima panda and Sushanth K Behra (2006) Indian jornal of Traditional knowledge vol 5(4), October, -p 519-528.

5.Prashanth p and Vidya sagar (2008) Indian journal of Traditional knowledge vol 7(2),April, 273-276.

6. Mirutse Giday, Zemede Asfaw, ZerihunWoldu (2009)Medicinal plants of the Meinit ethnic group of Ethiopia: An ethnobotanical study.J. Ethnopharmacol.

7.Raju sathiyaraju,Ariyan sarvalingam Arul balachandran,Rama koti redddy (2015) Diversity of Ethnomedical plants in Bodamali hills eastern ghats Namakkal district Tamil nadu.Journal of plant science 2015;3(2);77-84.

8.Amit panday and Shwetha sing (2017) Ethnobatonical evidences of common wild medicinal herbs existing on Delhiridge a chick list Journal of medicinal plants studies 2017;5(5);46-60.
9. Jayaraman I, Ghanim A, Khan HA. (1980). A new prenylated flavanone [7-methylglabranin] from*Tephrosia villosa*. *Phytochemistry* 19.
10. Rao PP, Srimanarayana G. (1981) .Tephrinone—a new flavone from *Tephrosia villosa*. *Curr. Sci* 50,319-320.
11. Prashant A, Krupadanam GD. (1993). A new prenylated dehydrorotenoid from *Tephrosia villosa* seeds.*Journal of natural products* 56, 765-766.
12.Prashant A, Krupadanam GL. (1993). Dehydro-6-hydroxyrotenoid and lupenone from *Tephrosia villosa*. *Phytochemistry* 32, 484-486.
13. David Krupadanam GL, Sarma PN, Srimannarayana G, Subba Rao NV. (1977). New C-6 oxygenatedrotenoids from *Tephrosia villosa* -- villosin, villosone, villol and villinol *Tetrahedron Letters*18, 2125-2128.
14. Ganapaty S, Lakshminarayana K, Lakshmi P, Thomas PS. (2009). *Asian Journal of Chemistry* 21,
1007-1010.
15.Ganapaty S, Nyamathulla S, Srilakshmi GVK, Prasad KVNMR. (2008). Chemical and Antimicrobial Studies of the Roots of *Tephrosia villosa* (L) Pers.*Asian Journal of Chemistry* 20, 4498-4502.

16. Madhusudhana J, Reddy RN, Reddy BAK, Reddy MVB, Gunasekar D, Deville A, Bodo B. (2010).Two new geranyl flavanones from *Tephrosia villosa*. *Natural Product Research* 24, 743-749.
17..Ganapathy S,Nymathulla S, G V K Srilakshmi (2008) Chemical and antibacterial studies of roots of tephorsia villosa (l)per.Asian journal of chemistry vol 20,No 6 4498-4502 (2008
18.Ramadhani S O Nando, Zakario H Mbmawbo(2011) BMC Complementary and alternative medicine 2011
19.Sufiyan ahmed . B R Balakrishanan (2009),Anti diabetic activity of leaves of Tephrosia villosa pers.in alloxan induced diabetic rats Journal of pharmacy Research vol 2,Issue 3 528-532,
20.D K Patel, R Kumar, D Haloo(2012),Diabetes mellitus :An overview on its pharmacological aspects and reported medicinal plants having ant diabetic ,Asian journal of Tropical biomedicine,may;2(5) pp 411-420.
21.Varaprasad Bobbrala, Chandrashekhara K naidu(2012) Alternative approaches for the control of Sorgham pathogens using selected medicinal plants, Intech open acess book,
22..Odhong c, R G Wahane,Vaarst,Nalubwana(2014), In vitro anthelmentic effect of crude aquous extracts Tephrosia vogeli, Tephrosia villosa and carica papaya leaves and seeds African journal of Biotechnology, vol 13 (52),pp 4467-4672,20

23..Aparna surya mani,Yejela Rajendra Prasad(2005), Phytochemical and antioxidant activity and screening of chloroform leaf and aerial parts extract of Tephorsia villosa .
24..A Samsath begum, A Jamal abdual Nasser(2017), Corrosion inhibition by aqueous extract of Tephorsia villosa leaves. World journal of Pharmaceutical research.vol 6, issue 17,1072-1100,