

**EFFECT OF *Tylophora indica* ON DIABETE IN DIABETIC MICE**

**Shahla Najafi\***

Department of Biology, Faculty of Science, University of Zabol, Zabol, Iran.

**Summary**

*Tylophora indica* (Burm. f.) Merr is a medicinal member belongs to the tribe Marsdenieae of the sub-family Asclepiadoideae and family Asclepiadaceae. Traditionally, some medicinal member belongs to the tribe Marsdenieae has been used in treatment of diabetes. The present study explores the anti-diabetic potential of *T. indica* in diabetic Balb/c mice. Qualitative test of gymnemic acid from the stem and leaf of *T. indica* were carried out. Diabetic Balb/c mice were treated with the alcoholic extracts of *T. indica*. Blood glucose level in diabetic Balb/c mice was estimated. The leaf and stem of this plant gave positive test for gymnemic acid. Results indicated that *T. indica* showed hypoglycemic activity. These results suggested that *T. indica* can be used as an anti-diabetic.

**Key Words:** Anti-diabetic; *Tylophora indica*

**\*Corresponding Author:**

Shahla Najafi  
Department of Biology,  
Faculty of Science,  
University of Zabol,  
Zabol, Iran  
E.mail: najafi\_sh2003@yahoo.com

**Introduction**

Diabetes is a consequence of abnormalities in the blood levels of insulin, the hormone that converts blood sugar into energy. Adult-onset diabetes is caused by the body's inability to adequately process of insulin. Today it is known as Type II diabetes, non-insulin-dependent diabetes mellitus (NIDDM) and stable diabetes.

Type I diabetes or juvenile diabetes results from an insulin shortage. Type I diabetes is also called insulin-dependent diabetes mellitus (IDDM). The tribe Marsdenieae of the sub-family Asclepiadoideae and family Asclepiadaceae contains a number of medicinally important genera like *Gymnema*, *Bidaria*, *Tylophora* and *Wattakaka*. Traditionally, some medicinal member belongs to the tribe Marsdenieae has been used in treatment of diabetes. Thousands of years ago, Type II diabetes was treated with *Gymnema sylvestre* a medicinal member belongs to the tribe Marsdenieae . In ancient Indian texts, *Gymnema* is referred to as *Gurmar*, which means, “sugar killer” in Sanskrit. Antidiabetic effect of a leaf extract from *G. sylvestre* in non-insulin dependent diabetes mellitus patients, normal and diabetic persons and rat has investigated by various research workers. Notable among these are Warren (15), Yackzan (16,17,18,19). *G. sylvestre* leaves contain gymnemic acids, which are known to suppress transport of glucose from the intestine into the blood stream (1,2,8,11,12), therefore, it is useful in lowering blood sugar, lowering blood cholesterol levels, balancing insulin levels and also for promoting weight loss. Recently, hypoglycemic potential of *Bidaria khandalense* (Sant.) Jagtap and Singh in diabetic Balb/c mice has investigated and results indicated that *B. kandalense* showed hypoglycemic activity like that of *G. sylvestre* (11). *Tylophora indica* (Burm. F.) Merr. (= *T. asthamatica* Weight) is a medicinal member belongs to the tribe Marsdenieae. *T. indica* has recently been included as one of the important drug from natural source for the treatment of respiratory diseases. Traditionally, *Tylophora indica* has been used in treatment of asthma, dermatitis and rheumatism. According to Chopra et al. (5), the powdered leaves, stem and root contain 0.2 - 0.3 per cent (up to 0.42-0.46 %) of alkaloids. Tylophorine and tylophorinine are important alkaloids encountered and the percentage is not affected by seasonal variations (6). The effect of the alcoholic extract of *Tylophora indica* on weight of the adrenal glands and its functional activities and pituitary adrenal axis was studied on normal, unilaterally adrenalectomised, dexamethasone treated and hypophysectomised male albino rats. The extracts showed stimulation of adrenals as indicated by increase in weight and decrease in cholesterol and vitamin C. The plasma steroid level was increased but skin hydroxyproline level findings were not conclusive. From this result it may be concluded that *Tylophora indica* acts as direct stimulation of adrenal cortex (14). However, no literature is available on its chemical constituents and anti-diabetic potential of this plant. Thus, we selected *Tylophora indica* of the tribe Marsdenieae to evaluate for its anti-diabetic action in diabetic Balb/c mice.

### Materials and Methods

**a) Material :** Streptozotocin and Dimethyl sulfoxide (DMSO) were obtained from Sigma chemicals and Accu-chek blood glucose analyzer was a product of Roche.

The plants specimens were collected from various places of Maharashtra State (India) such as Ambaghat, Amboli, Mahabaleshwar and Dapoli Efforts were made to collect the plants when they started flowering and fruiting for the correct botanical identification. The plant material was brought to the laboratory and identified with the help of flora of Maharashtra State (13), Fasicals of flora of India (9) and Flora of British India (8).

**b) Animals:** The Experimental Animal Facility at the National Centre for Cell Science, India provided all the animals. Male Balb/c mice 6-8 weeks old were made diabetic by intraperitoneal injection of streptozotocin (180 mg/kg body weight) freshly dissolved in chilled sodium citrate buffer (pH 4.5). Mice showing blood glucose above 200 mg/dL were taken for further experiments. They were kept under 12h in light and 12h in dark conditions at 25°C and fed them with ad libitum during the experiment (4).

**c) Phytochemical screening:** In the present investigation qualitative test of gymnemic acid from the stem and leaf of *T. indica* were carried out (12).

#### **c - 1) Qualitative test of Gymnemic acid:**

##### **Procedure:**

Sample Preparation: Take 1 gm of stem or leaf powder of plant samples of each studied material and mixed in 5 ml methanol (Conc. 200mm or 200µg/µl), Sonicate the mixture, filter the extract and filtrate was used for application.

Stationary Phase: Percolate Silica Gel TLC Plate (20x10cm Merc No. 5554).

Mobile Phase : N butanol : Methanol : Water.

3 : 1 : 1

Developing distance : 80mm.

Tan Saturation : 10 min.

Scanning Wavelength : 580 nm.

Spraying reagent : raniline sulphuric acid.

Rf : 0.61.

Standard gymnemic acid :1µg/µl = 5µl i.e 1%

**d) Test for hypoglycemic potential:** Diabetic Balb/c mice were randomly divided into 6 groups (each having 5 animals) to examine the glucose-lowering property of *T. indica*. All extracts were administered orally at a dose of 50 mg/kg body weight. An effect of vehicle administration DMSO was also determined by acute oral gavage. Blood was collected from tail vein immediately prior to and 24h after administration of the extracts for blood glucose analysis by Accu-chek blood glucose analyzer from Roche (3).

### Results

**Phytochemical screening:** Results on High Performance Thin Layer chromatography (HPTLC) indicated that the leaf and stem of *T. indica* gave positive test for gymnemic acid.

**Hypoglycemic study:** Results indicated that *T. indica* showed hypoglycemic activity (Table No.1). The hypoglycemic activity is first time reported in *T. indica* and this can be used as a substitute an anti-diabetic to *G. sylvestre*. These results suggested that *T. indica* can be used as an anti-diabetic. Thus, the results of this investigation will be helpful for alternative sources of medicine in Ayurvedic, Homeopathic, Unani, Allopathic systems of medicine and Pharmaceutical industries.

**TABLE NO. 1: SCREENING OF PLANT EXTRACTS FOR THEIR HYPOGLYCEMIC POTENTIAL**

Plant	Blood glucose at 0 h		Blood glucose at 24 h		% change at 24h
	Mean	S. E.	Mean	S. E.	w.r.t. 0h
Name of the Plant & vehicle administration					
(1) <i>T. indica</i>	463.7500	70.1824	517.0000	62.9524	11.4800
(2) DMSO Dimethy sulfoxide (DMSO)	287.0000	39.5095	344.6667	54.7002	20.0900
% change = $\frac{\text{final level} - \text{initial level}}{\text{initial level}} \times 100$					
Values represent mean ± SE					

### References

1. Anonymous. Pharmacopoeia of India. Government of India Ministry of Health Manager Publications Delhi 1<sup>st</sup> Edn 1955;370 and 864.
2. Anonymous. The wealth of India. CSIR Pub. New Delhi 1972; III : 41 - 43.
3. Bhonde R, Shukla R, Barve V, et al. Synthesis structural properties and insulin – enhancing potential of bis (quercetinato) oxovanadium (IV) conjugate. Bioorganic and Medicinal Chemistry Letters 2004;14 : 4961 - 4965.
4. Chattopadhyay P R. A comparative evaluation of some blood sugar lowering agents of plant origin. J Ethnopharmacol 1999;67 (3) : 367 - 372.
5. Chopra RN, Nayar SL, Chopra IC. Glossary of Indian Medicinal Plants. C S I R Pub New Delhi India 1956; 94.
6. Gopalakrishnan C, Shankaranarayan D, Kameswaran L. Pharmacological investigations of tylophorine the major alkaloid of *Tylophora indica*, *Indian J. Med. Res.* 1979; 69 : 513 - 20.
7. Gopalakrishnan C, Shankaranarayan D, Nazimudeen SK. Effect of tylophorine, a major alkaloid of *Tylophora indica* on immunopathological and inflammatory reactions, *Ind. J. Med. Res.* 1980; 71 : 940 - 8.
8. Hooker J D. Flora of British India. 1872;IV : 28-33.
9. Jagtap AP, Singh NP. Fascicles of Flora of India. The Director, Botanical of India 1999;24 : 1-6, 82-91, 62-71.
10. Nadkarni A K. Indian Materia Medica. Bombay India Popular Prakashan 1927; 1:1252.
11. Najafi S, Deokule SS, Shitole MG. EFFECT OF *Bidaria khandalense*, *Gymnema sylvestre* AND *Wattakaka volubilis* ON DIABETE IN DIABETIC MICES.
12. Passera C A, Pedrotti, Ferrari G. Chromatography. 1964;14 : 289.
13. Singh N P, Lakshminarasimhan P, Karthikeyan S, et al. Flora of Maharashtra State. Dicotyledones. The Director, Botanical Survey of India. 2001;334 – 342 : 362-364.
14. Udupa A L, Udupa S L, Guruswamy M N. The possible site of anti-asthmatic action of *Tylophora asthmatica* on pituitary-adrenal axis in albino rats, Department of Pharmacology, Kasturba Medical College, South Karnataka, India. *Planta Med*, 1991; 57 (5) : 409 -13.

15. Warren R P, Warren R M, Weniger M G. Inhibition of the sweet taste by *Gymnema sylvestre*, *Nature*. 1969; 7 : 223 (20) : 94 - 95.
16. Yackzan K S. Biological effects of *Gymnema sylvestre* fractions, II. Electrophysiology, effect of gymnemic acid on taste receptor response, *Ala J. Med Sci*. 1969; 6 (4) : 455 - 463.
17. Yackzan K S. Biological effects of *Gymnema sylvestre* fractions 3, Electrophysiology in vitro gastric membrane findings, *Ala J. Med Sci*. 1970; 7: 77-79.
18. Yackzan K S, Yourself M K, Dill D B. Comparative electro cardiography in Lizards : effects of temperature and *Gymnema sylvestre* fractions, *Comp. Biochem. Physiol*. 1972; 43 (2) : 381 - 389.
19. Yackzan K S, Stoll P J. Sensory effects of *Gymnema sylvestre* : Implication of mass action and chemical kinetics, *Ala J. Med Sci*.1977; 14 (2) : 156.
20. Yoshikawa K, Amimoto K, Arihara S, et al. Structure studies of new antisweet constituents from *Gymnema sylvestre*. *Tetrahedron Letters* 1989;30 (9) :1103 -1106.
21. Yoshikawa K, Arihara S, Matsuura K. A new type of antisweet principles occurring in *Gymnema sylvestre*. *Tetrahedron Letters* 1991;32 : 789 - 792.