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IN VITRO STUDY OF METHANOLIC EXTRACTS OF *DODONAEA VISCOSA*. LINN AND *WRIGHTIA TINCTORIA* R. BR. ON GLUCOSE UPTAKE BY ISOLATED RAT HEMI- DIAPHRAGM

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ABSTRACT

In the present investigation, the methanolic extract of leaves of *Dodonaea viscosa* (D.viscosa) and pods of *Wrightia tinctoria (W. tinctoria)* were evaluated for antidiabetic activity. The antidiabetic activity was studied using the Glucose uptake by isolated rat hemi-diaphragm *in vitro* model. The value of glucose uptake by rat hemi-diaphragm for *D. viscosa* was 13.80 ± 0.1697 and for *W. tinctoria* was 9.384 ± 0.3944 as compared to control (5.34 ± 0.12) and insulin 15.45 ± 0.12 in mg/g/min. The results strongly suggest that *D. viscosa* will be alternative choice for the treatment of diabetes mellitus caused in the consequences of resistance to stimulatory effect of insulin on Glut-4 protein.

Key words: Antidiabetic, Rat hemi-diaphragm, *Dodonaea viscosa*, *Wrightia tinctoria.*

INTRODUCTION

Dodonaea viscosa linn. jacq is a shrub belonging to family Sapindaceae. The centre of origin of *D. Viscosa* is believed to be Australia but it occurs throughout the tropics and subtropics widely distributed in temperate region of Mexico, Newzeland, India, Florida and South America. It is a traditional medicine worldwide, administered orally to treat great variety of ailments[1]. Stems are used as fumigants to treat rheumatism. Stems, leaves and

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seeds are used to treat fever, sore throats and cold. Leaves are used to treat aches and can be used as antispasmodic agent[2]. Leaves and roots are used as pain killer to soothe tooth ache and head aches[3]. A large number of constituents have been isolated from the extracts of the whole herb leaves, flowers, seeds, and bark. Previous chemical studies on this species resulted in the isolation and characterization of several flavonoids[4], diterpenoid acids[5], biologically active saponins[6], p-coumarin acid ester[7], Sterols[8] Tannins from aerial parts of *D.viscosa*[9]. More recently Wollenweber et al.[10] isolated relatively large concentrations of flavonoids from leaf extract of *D. viscosa*.

Wrightia tinctoria Roxb. R.Br belonging to family Apocynacea is a small deciduous tree generally grows upto 1.8 m tall and sometimes upto 7.5 length, distributed all over India[11,1]. This plant is externally used indigenously in Indian system of traditional medicine as a remedy for various ailments as stomachic, skin diseases, antidiarrhoel, antihaemorrhagic[12], almost every part of plant is useful-leaves pungent chewed for relief from tooth ache[1], Bark and seeds are antidysenteric, antidiarrhoel and antihaemorrhagic[13]. Oil emulsion of leaves and pods is used to treat psoriasis[14,15]. Five flavanoids from leaves indigotin, indrubin, isatin, tryptanthin and rutin were isolated[16]. Ursolic acid and iso ricinolic acid has been also isolated from the seed pods and seed oil[17]. The plant is also reported for its antimicrobial activity, wound healing, hepatoprotective activity[18-21].

Diabetes mellitus can be defined as a group of metabolic disease characterized by chronic hyperglycemic resulting from defects in insulin secretion, insulin action, and impaired function in carbohydrate lipid and protein metabolism. Skeletal muscle is a major tissue for blood glucose utilization and a primary target tissue for insulin action. Insulin increases glucose uptake in skeletal muscle by increasing functional glucose transport molecules (GLUT-4) in the plasma membrane. Glucose transport in skeletal muscle can also be stimulated by contractile activity. Free radical impairs insulin-stimulated GLUT-4 translocation and exerts an inhibitory effect on muscle contractility that is major pathological feature of diabetes. The estimation of glucose content in rat hemi-diaphragm is a commonly employed and reliable method for *in vitro* study of peripheral uptake of glucose. From ancient period people are using medicinal plants for the treatment of diabetes and WHO estimates that 80% of the population presently uses herbal medicines for primary health care[22]. Antidiabetic plant has the ability to restore the functions of damaged pancreatic tissue by increasing the insulin or inhibiting intestinal absorption of glucose[23]. Preclinical experiments should be initially carried out when possible with *in vitro* studies to explore and advance in mechanism of action of a natural products. The present study was

aimed at investigating the antidiabetic effect of *Dodonaea viscosa. Linn* and *Wrigtia tinctoria.*

EXPERIMENTAL

Materials and method

Plant material

Fresh leaves of *Dodonaea viscosa* and pods of *Wrightia tinctoria* were collected from Kesara, Ranga Reddy district, Hyderabad. The plants were authenticated by Dr. Bhadraiah, Department of Botany, Osmania University, Hyderabad. The voucher specimen was deposited in herbarium of the University for future Reference.

Preparation of extract

The dried plant material was chopped, coarsely powdered and extracted using methanol by maceration. The extract was then concentrated to dryness under reduced pressure and controlled temperature to yield a semi solid mass.

Animals

Healthy adult albino rats of Wister strain weighing 150-200 g were obtained from NIN, Hyderabad. The animal house was well ventilated and animals had 12 ± 1 hour and day and night schedule with temperature between 15-20 ± 5^{o}C. The animals were housed in standard polypropylene hygienic cages (three animals per cage). The animals were fed with rat pellet feed. The current work was carried out after approval by our institutional ethical committee.

Glucose uptake by isolated rat hemi-diaphragm

Glucose uptake by rat hemi-diaphragm was estimated by the methods described by Walass[24] and Chattopadhyay[25] with some modification. Albino rats of either sex weighing between 160-200 gm were selected. The animals were maintained on a standard pellet diet (water ad libitum), and fasted overnight. The animals were sacrificed by decapitation and diaphragms were dissected out quickly with minimal trauma and divided into two halves. The hemi-diaphragms were then rinsed in cold Tyrode solution (without glucose) to remove any blood clots and were placed in small culture tubes containing 2 mL Tyrode solution with 2% glucose and incubated for 30 minutes at 37^{o}C in an atmosphere of 100% oxygen with

shaking. Four sets containing five numbers of graduated test tubes (n = 5) each, were taken as follows:

Group 1: Served as control which contained 2 mL of Tyrode solution with 2% glucose and 2 mL of distilled water.

Group 2: Contained 2 mL of Tyrode solution with 2% glucose, regular insulin (Biocon) 0.62 mL of 0.4 units per mL solution and 1.38 mL of distilled water.

Group 3: Contained 2 mL of Tyrode solution with 2% glucose, 0.6 mL of *D. viscosa* and 1.4 mL of distilled water.

Group 4: Contained 2 mL of Tyrode solution with 2% glucose, 0.6 mL of *W. tinctoria* and 1.4 mL of distilled water.

Two diaphragms from the same animal were not used for the same set of experiment. Following incubation, the hemi-diaphragms were taken out and weighed. The glucose content of the incubated medium was measured by GOD-POD method. The uptake of glucose was calculated in mg/g of most tissue/ 30 min. Glucose uptake per gram of tissue was calculated as the difference between the initial and final glucose content in the incubated medium.

Statistical analysis

The data obtained was statistically analyzed by one way ANOVA and expressed as mean ± S.E.M, followed by dunnett's test using computerized graph pad InStat version 3.06 graph pad software.

RESULTS AND DISCUSSION

The experiment focused on exploring the competence of methanol extract of *D. Viscosa* and *W. Tinctoria* for the correction of diabetes to substantiate folkore claim. *D. Viscosa* extract enhanced the uptake of glucose by isolated rat hemi-diaphragm significantly ($p < 0.01$) than the *W. Tinctoria* and was found to be nearly effective as that of insulin. The control group of glucose uptake by rat hemi diaphragm corresponds with the extraction of glucose content in rat hemi-diaphragm.

The effect of the methanolic extract of *D. Viscosa* and *W. Tinctoria* on glucose uptake by isolated rat hemi diaphragm is illustrated in Table 1 and Fig. 1.

Table1: Effect of *D. viscosa* and *W. tinctoria* on glucose uptake by isolated rat hemi-diaphragm

S. No.	Group	Glucose uptake mg/mL/30 min.
1	Control	5.34 ± 0.12
2	Insulin	15.458 ± 0.12**
3	D. Viscosa	13.802 ± 0.16**
4	W. Tinctoria	9.384 ± 0.39**

Values are mean ± SEM; N = 5, * p < 0.05**** p < 0.01***.***p < 0.001 as

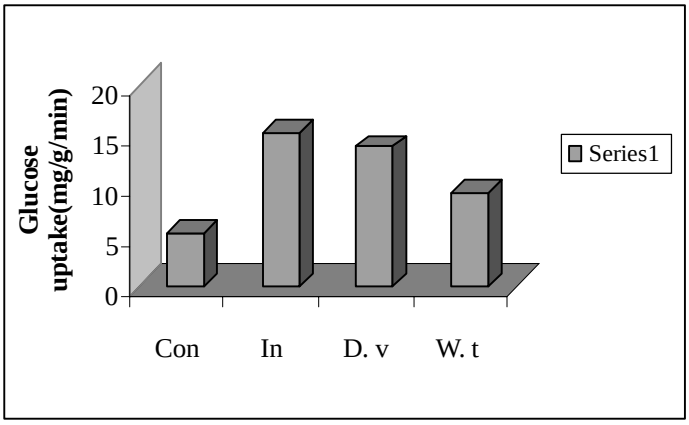

Fig. 1: Effect of *D. Viscosa* and *W. tinctoria* on glucose uptake by isolated rat hemi diaphragm

REFERENCES

1. K. R. Kirtikar and B. D. Basu, Indian Medicinal Plants, 2nd Edition, Jayyed Press, Delhi (1975) p. 1581.

2. A. S. Rojas, H. Cruz, Ponce-Monter and R. Mata, Smooth Muscle Relaxing Compounds from *Dodonea Viscose*, Planta Medica., **62**, 154-159 (1996).

3. A. B. Cribb and J. W. Crib, Wild Medicine in Australia, Collins, Sydney (1981) p. 228.

4. K. Sachdev, D. K. Kulashreshtha and Aliarin a New Flavonid from *Dodonea Viscose*, Indian J. Chem. Section B Org. Chem. Including Medic. Chem., **21**, 798-789 (1982).

5. K. Sacdev and D. K. Kulashreshtha, Dodonic-Acid a New Diterpinoid from *Dodonea-Viscosa*, Planta Medica, **50**, 448-449 (1984).

6. H. Wagner, C. Ludwig, L. Grotjahn and M. S. Y. Khan, Biologically Active Saponins from *Dodonea-Viscosa*, Phytochemistry (Oxford), **26**, 697-702 (1987).

7. Rachel Mata and Jose Luis Contreris et al., Studies on Mexican Plants used in Traditional Medicine, New Secondary Metabolites from *Dodonaea Viscosa*, J. Natural Prod., **54(3)**, 913-917 (1991).

8. H. Y. Hsu, Y. P. Chen and H. Kakisawa, Phytochemistry, **10**, 2813 (1971).

9. K. N. S. Sastry and Y. Nayudamma, Leather Sci., **13**, 174 (1996).

10. E. Wollenweber and J. N. Roitman, New Reports on Surface Flavonoids from *Chamaebatiaria (Rosaceae), Dodonea (Sapindaceae), Elscholtzia (Lamiaceae) and Silphium (Asteraceae)* Natural Products Communications, **2**, 385-389 (2007).

11. Anonymous, The Wealth of India Raw Material, **Vol. X**, Publication and Information Directorate CSIR, New Delhi, India (1976) p. 588.

12. K. M. Nadkarni, Indian Materia Medica, **Vol. I**, Popular Prakashan, Bombay, (1976) p. 1296.

13. V. P. Singh and S. K. Sharma, Pharmacognostical Studies on Wrightia Tinctoria Bark, Indian Drugs, **17**, 7-10 (1980).

14. K. Mitras, J. Seshadris, M. Venkataranganna and S. Vandgopumadhvan, Reversal of Paraleratosis, A Feature of Psoriasis by *Wrightia Tinctiria* (in Emulsion) Histological Evaluation (1998).

15. J. R. Krishna Moorthy and S. Ranganathan, Antipityrosporum Ovale Actiivity of a Herbal Drug Combination of *W. Tinctoria* and *Hibiscus Rosasinensis*, Lnd. J. dermatol, **45(3)**, 125-126 (2000).

16. A. V. Muruganadam, S. K. Bhattacharya and S. Ghosal, Indole and Flavonoid Constituents of *Wrightia Tinctoria* and *W. Tomentosa* and *W. Coccinea*, Indian J. Chem., **39B(2)**, 125-131 (2000).

17. I. Ahmad and M. S. F. Lie ken Jie, Oleochemicals from Isoricinoleic Acid (*Wrightia Tinctoria* Seed Oil), Ind. Eng. Chem. Res., **47**, 2091-2095 (2008).

18. R. Dang, J. S. Sabitha and B. G. Shivanand, Anti-Microbial Activity of Herbs used in Psoriasis, The Pharma Review, **9**, 31-32 (2005).

19. V. P. Veerapur, M. B. Palkar, H. Srinivasa, M. S. Kumar, S. Patra and P. G. M. Rao, J. Natural Remedies, **4(2)**, 155-159 (2004).

20. V. Chandrashekar and A. N. Nagappa, Hepatoprotective Activity of *Wrightia Tinctoria* (Roxb) in Rats, Indian Drugs, **41(6)**, 366-370 (2004).

21. S. Pritam and B. Sanjay, Antibacterial and Anti Fungal Activity of Extracts of Woody Stem of *Wrightia Tinctoria* R. Br, Int. J. Pharma. Recent Res., **1(1)**, 18-21 (2009).

22. L. R. Atmakuri and S. Dathi, Current Trends in Herbal Medicines, J. Pharm. Res., **3**, 109-113 (2010).

23. N. Malviya, S. Jain and S. Malviya, Antidiabetic Potential of Medicinal Plants, APPHA, **67**, 113-118 (2010).

24. E. Walaas and O. Walaas, Effect of Insulin on Rat Diaphragm Under Anaerobic Conditions, J. Biol. Chem., **195**, 367-373 (1952).

25. R. R. Chattopadhyay, S. K. Sarkar, S. Ganguly, R. N. Banerjee and T. K. Basu, Effect of Leaves of *Vincarosea* Linn. on Glucose Utilization and Glycogen Deposition by Isolated Rat Hemidiaphragm, Indian J. Physiol. Pharmacol., **36**, 137-138 (1992).

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