EUROPEAN JOURNAL OF BIOMEDICAL AND PHARMACEUTICAL SCIENCES
http://www.ejbps.com

ISSN 2349-8870
Volume: 9
Issue: 11
177-180
Year: 2022

IN VITRO ANTIDIABETIC ACTIVITY OF ETHANOL EXTRACT OF EEMBELIA RIBES BY GLUCOSE UPTAKE ASSAY BY PERIPHERAL TISSUES

Amir Sohel[1]*, Jha Aditya G. K.[1], Ramesh C.[1], Sowmya B. A.[1] and Pinkey Rawal[2]

[1]Department of Pharmacology, East West College of Pharmacy, Bangalore.
[2]Department of Pharmacology, East West College of Pharmacy, Bangalore.

***Corresponding Author: Amir Sohel**
Department of Pharmacology, East West College of Pharmacy, Bangalore.

Article Received on 06/09/2022 **Article Revised on 27/09/2022** **Article Accepted on 16/10/2022**

ABSTRACT
The present research study was conducted to determine the mechanism of action of *Embelia ribes* for its antidiabetic properties. **Materials & Methods**: The authenticated leaves of plant *Embelia ribes* were dried and powdered. The powdered drug was defatted with petroleum ether and subjected to ethanol extraction. The ethanol extract of Embelia ribes was subjected to preliminary phytochemical investigation. The glucose up takes by rat hemi-diaphragm (skeletal muscle) model was also used evaluate potentials of the ethanol extract of *Embelia ribes* to enhance utilization of the blood glucose by peripheral tissues. The overnight fasted albino rat was sacrificed and dissected to hemidiaphragm. Weighed quantity of skeletal muscle was incubated with glucose in Tyrode solution for 30 mins at 37^{o}C and the amount of glucose utilized by the tissue was determined. **Results**: The ethanol extract of *Embelia ribes* significantly increased the glucose utilization by skeletal muscle which its shows potency to sensitize the insulin. **Conclusion**: The results of the presents investigation recommends that, one of the mechanism of ethanol extract of *Embelia ribes* for antidiabetic activity is reduction of insulin resistance.

KEYWORDS: Antidiabetic activity, *Embelia ribes*, Hemidiaphragm, Glucose utilization.

1.0 INTRODUCTION
Diabetes mellitus develops due to disturbances of metabolism in the body as result of absolute or relative absence insulin or insulin resistance which ultimately leads to alterations in metabolism of nutrients carbohydrates, amino acids and fats.[1] According to survey made by World Health Organization (WHO), about 220 million people throughout world will have diabetes mellitus and India would be diabetic capital of the world by 2020. Hence there is always scope for the development of anti-diabetic drugs due to its high prevalence and long term complications of disease.[2] The insulin a peptide hormone produced from recombinant DNA technology is used in insulin dependent diabetes mellitus (Type 1 or IDDM) and oral hypoglycemic drugs are used in non-insulin dependent diabetes mellitus (Type 2 or NIDDM) to bring hyperglycemic to euglycemic condition in individuals. Although there is availability of several pharmacological agents for the management of diabetes mellitus, still there is no truly satisfactory drug for its effective management with last side effects. Hence identification and development of newer therapeutic agents remains highly desirable.[3] In view of the toxicities effects and adverse reactions associated with the therapy using presently available oral hypoglycemic drugs and insulin, searching for more potent and less toxic hypoglycemic drug from plant origin is under pipeline throughout the world since herbal medicine play essential role in this segment due to their minimum side effects.[4] Since ancient period Ayurveda physicians Charka and Sushruta had mentioned the usefulness of several medicinal plants for the effective management diabetes with fewer side effects in Ayurveda, the traditional medicinal system of India. Herbal remedies for diabetes mellitus constituting of plant substances, either a single agent or in combination with other drugs, which are considerably safe and free from adverse reactions compared to synthetic agents.[5]

Among various approaches for the management of diabetes mellitus such as stimulation of insulin release, inhibition of intestinal glucosidase and enhancement of glucose utilization by the tissues are important.

Embelia ribes is an important medicinal plant that is used in a long period of time as herbal remedy to treat various diseases and ailments.[6,7] Different parts of the plant such as the stem, leaves, roots, seeds and barks are widely accepted worldwide in traditional setting and used as various form of remedies such as diuretic, astringent, anti-inflammatory, antibacterial, antihelmintic, etc. Modern researchers however showed that the various plant extracts has exhibited many

potential as antibacterial, anti-fungal, anti-inflammatory, analgesic, antitumor and contraceptive ability.[8,9] These attributes are both related to an array of phytochemicals that are embedded within the plant parts.[10,11,12] Recently the extract of the plant is reported for antidiabetic activity in animal models.[13] Hence, the present study is designed to test effect of extract on glucose utilization by hemidiaphragm to determine the mechanism of action of the ethanol extract of *Embelia ribes* for antidiabetic potentials.

2.0 MATERIALS AND METHODS

Plant material: The areal part of *Embelia ribs* was collected from Foundation for Revitalization of Local Health Traditions (FRLTH) No.74/2, Jarakabande Kaval, Post Attur, Via Yelahanka, and Bangalore, Pin Code: 560106 Karnataka, INDIA. The plant was authenticated by Dr. Rama Rao, Scientist, Regional Ayurveda Research Institute for Metabolic Disorders.

2.1 Preparation of the ethanol extract

The leaves of the plant are collected and dried under shade. The dried leaves are then powdered and the coarse powder will be defatted with petroleum ether. The defatted powdered drug will be subjected to ethanol extraction in soxhlet apparatus for 48 hours and the marc left over will be subjected to aqueous extraction using chloroform water.[14]

2.2 Preliminary phytochemical investigation

The preliminary phytochemical investigation for the ethanol (EEEER) of *Embelia ribes* was conducted as per procedure prescribed by Khandelwal.[15]

2.3 Evaluation of in vitro antidiabetic activity of extract of *Embelia ribes*

2.3.1 Glucose uptake by isolated rat hemidiaphragm

The utilization of glucose by skeletal muscle of rat(hemidiaphragm) was assed according to methods described in previous investigations.[16] The study consisting of four categories, with each group containing 6graduated test tubes, were regarded as follows:

- **Category I:** Consists of 10 mL of 4% glucose in Tyrode solution.
- **Category II**: Consists of 10 mL of 4% glucose in Tyrode solution and regular insulin suspension (1IU).
- **Category III**: Consists of 10 mL of 4% glucose in Tyrode solution and 1.38 mL of EEER (0.1% v/v).
- **Category IV**: Consists of 10mL of 4% glucose in Tyrode solution and regular insulin (0.62 mL of 0.4 U/mL) solution and 1.38 mL of EEER (0.1% v/v)

The quantities of all the assay tubes were make up to 4 mL individually by mixing distilled water to make up the total volume of the assay tubes. A total of healthy albino rats of wistar species were kept fasting for whole night and sacrificed under light anesthesia. The diaphragms of experimental animals were quickly cutted with little damage and splitted into 2equal halves. For the same set of study, two diaphragms from the same animal were not used. About xix diaphragms were utilized in every category of study. The collected skeletal muscles (diaphragm) were kept in assay tubes and incubated at 37^{O}C for about 30 minutes in an atmosphere constitutes 100% oxygen and were shuddered at a speed of 140 CPM. The amount of utilization of glucose per every gram of tissue was determined as the difference between the concentrations of starting and final glucose in the incubated medium.[17,18]

3.0 RESULTS

3.1 Preliminary phytochemical investigation

The percentage yield of the EENN was found to be 9.24 % w/w. The preliminary phyto-chemical investigation for the methanol extract of *Nymphaea nauchali* reveals the presence of poly phenols, flavonoids, tannins, steroids, alkaloids and carbohydrates.

3.2 Effect on peripheral glucose uptake

In the present study, the methanol extract of *Embelia ribes* significantly increased utilization of glucose by rat hemidiaphragm and the effect was comparable to standard agent Insulin. The combination of TPME with insulin has shown synergistic property. The results clearly indicate that administration of insulin and TPME alone for 30 minutes caused a significant enhancement in glucose absorption by 3.37- and 2.80- times, respectively. Addition of both insulin and TPME to the incubation media exhibited the rate by 3.55-times, an elevation of utilization of glucose hemidiaphragm of rat when compared with untreated control animals but there was no much significant elevation compared insulin alone treated group [Table No 5]. The glucose utilization by rat skeletal muscle was considerably large in all the categories examined when collate with the vehicle control.

Table No 5.3: Effect of EEER on glucose uptake by isolated rat hemi-diaphragm.

S.No	Glucose uptake for 30 mins (mg/g)
Control	78.234±1.66
Insulin	264.11±2.88**
EEER	219.69±1.23**
EEER + Insulin	278.44±2.94**

Values are mean ± SEM (n Z 6).
** $p < 0.01$ as compared with control

4.0 DISCUSSION

Diabetes mellitus is a metabolic, multifactorial and devitalizing disease with increasing occurrence in the entire world which may leads to various complications such as multi organ failures, peripheral neuropathy, retinopathy, nephropathy, hyperlipidemia and various cardiovascular disorders.[19,20] One of the novel therapeutic approach for management of diabetes mellitus is enhancement of glucose by peripheral tissues is very important mechanism. The skeletal muscle comprises about 30-40% of the total quantity of body and hence it can be one of the most major target tissues

for the activity of insulin which enhances the utilization of glucose at the peripheral level. It is a well understood that insulin and anti-diabetic drugs stimulate glucose utilization by peripheral cells and tissues.[21] The major finding of the present study is that EEER have significant action similar to insulin as witnessed by the stimulation of glucose utilization from the rat's hemidiaphragm, which constitutes muscle tissue that are essential tissues of insulin regulated glucose discharge. The EEER considerably enhanced the uptake of glucose by isolated rats muscle hemidiaphragm and is observed to be less potent than insulin. It seems that EEER has action on peripheral tissues and results of normal group of glucose utilization by rat peripheral tissue corresponds with those of earlier findings.[22] In the present study, the EEER exhibited its potency to counter insulin resistance by increasing the utilization of glucose by peripheral tissues and extract also exhibited its potentials which may the possible mechanism of action for its benefit against diabetes.

5.0 CONCLUSION

The results of the presents investigation recommend that, one of the mechanism of ethanol extract of *Embelia ribes* for antidiabetic activity is reduction of insulin resistance. But further examination is necessary to isolate and estimate the specific components present in methanol extract of *Embelia ribes* that may be responsible for these beneficial properties to improve the health conditions connected with diabetes mellitus.

CONFLICT OF INTEREST

All authors are hereby declaring that there is no conflict of interest with respect to manuscript.

ACKNOWLEDGEMENTS

The authors of manuscript are thankful to The principal and management of East West College of Pharmacy, Bangalore for providing facilities to conduct this research work.

6.0 REFERENCES

1. G.B. Kavishankar, N. Lakshmidevi, S. Mahadeva Murthy, H.S. Prakash, S.R. Niranjana. Diabetes and medicinal plants-A review. Int J Pharm Biomed Sci., 2011; 2(3): 65-80.
2. Singh SK, Hawkins C, Clarke ID, Squire JA, Bayani J, Hide T, Henkelman RM, Cusimano MD, Dirks PB. Identification of human brain tumour initiating cells. Nature, 2004; 18; 432(7): 396-401.
3. Abidemi JA, Zahoor W, Girish M, Sadhana S, Flora RA, Naresh S, Olufunmilayo OA, Dilip MM. Anticancer activity of *Aristolochia ringens* Vahl. (Aristolochiaceae). J Trad Com Med, 2015; 5: 35-41.
4. Dhananjaya S and Aparna K. current status of cancer burden: Global and Indian scenario. Biomed Res J., 2014; 1(1): 1-5.
5. Nitesh K, Isha D, P. Vasanth R, B.S. Jayashree, Vipan P, S.N. Manjula, Seeja T, N. Gopalan K, C. Mallikarjuna R. Preliminary investigation of cytotoxic potentialof 2-quinolone derivatives using *in vitro* and *in vivo* (solid tumor and liquid tumor) models of cancer. Arabian J Chem., 2014; 7: 409–17.
6. Sankappa RU, A.M. Isloor, P. Shetty, K.S.R. Pai, H.K. Fun. Synthesis and in vitro biological evaluation of newpyrazole chalcones and heterocyclic diamides as potential anticancer agents. Arabian J Chem Volume, 2015; 8(3): 317–21.
7. Thirumal M, Kishore G, Prithika R, Das S, Nithya G. In vitro anticancer activity of *Tecoma stans* (L) ethanolic leaf extract on human breast cancer cell line (MCF-7). Int J Pharm Chem Biol Sci., 2012; 2(4): 488-93.
8. Valko M, Leibfritz D, Moncol J, Cronin MT, Mazur M, Telser J. Free radicals and antioxidants in normal physiological functions and human disease. Int J Biochem Cell Biol, 2007; 39: 44-84.
9. Aboul-Enein AM, Abu-Elalla F, Shalaby EA, El-Shemy HA. Traditional medicinal plants research in Egypt: studies of antioxidant and anticancer activities. J Med Plants Res., 2012; 6(5): 689-703.
10. Saldanha CJ, Singh BG, Leguminosae, In: Saldanha C.J. (Ed.) Flora of Karnataka Vol:I, Oxford and IBH, 1984; 495-499.
11. Mohan Maruga Raja MK, Neeraj Kumar S, and Mishra SH. A comprehensive review on *Nymphaea stellata*: A traditionally used bitter. J Adv Pharm Technol Res., 2010; 1(3): 311–9.
12. Manjeet S and Alok PJ. A review on genus *Nymphaea*: multi-potential medicinal plant. Asian J Pharm Edu & Res., 2017; 6(4): 1-9.
13. Sharanbasappa D, Veeresh PV, Satrasala N and Shivsharan BD. Antidiabetic activity of *Embelia ribes*, embelin and its derivatives: A systematic review and meta-analysis. Biomed & Pharmacother, 2017; 86: 195–204.
14. Kokate CK. Practical Pharmacognosy. Vallabh Prakashan New Delhi: 1994; 4: 110-1.
15. Kokate CK. Practical Pharmacognosy. Vallabh Prakashan New Delhi: 1994; 4: 110-1.
16. Khandelwal KR, Practical Pharmacognosy-techniques and experiments. Pune; NiraliPrakashan, 2000.
17. Walaas E and Walaas O. Effect of insulin on rat diaphragm under anaerobic conditions. J Biol. Chem., 1952; 195: 367-73.
18. Ajabnor MA and Tilmisany AK. Effects of *Trigonella feonumgraceum* on blood glucose levels in normal and Alloxan-diabetic mice. J.Ethnopharmacol, 1998; 22: 15-49.
19. Pamela CC, Richard AH, Denise RF. Type 1 Diabetes mellitus: Lippincott's Wlliams and wlikins, India, 1st edition, 1994: 336-7.
20. Satyanarayana U, Chakrapani. Insulin, glucose homeostasis and diabetes mellitus. Fundamentals of biochemistry Kolkata; Third edition, 2008: 678-680.
21. Melander A. Glucose uptake by hemidiaphragm. Diabet Med., 1996; 13: 143.

22. Cohen G, Heikkila KE. Generation of hydrogen peroxide, superoxide radical by 6-hydroxy dopamine, dialuric acid and related agents. J Biol and Chem., 1974; 49: 2447-52.