

**HYPOGLYCEMIC ACTIVITY OF SARACA INDICA LINN BY RAT
HEMIDIAPHRAGM METHOD**

Preethi .F*, Jennifer Fernandes

***Preethi mol Francis (Corresponding author)**

Lecturer

Rajiv Gandhi college of Pharmacy,

Trikaripur PO, Kasargod,671310

Kerala,India

Mob:-09497063973

preethiannjy@gmail.com

Dr. Jennifer Fernandes

Professor

NGSM Institute of Pharmaceutical Sciences,

Paneer, Derlakatte-575018

Mangalore,INDIA

Summary

Saraca indica Linn (Family: *Leguminosae*) is one of the most legendary and a sacred tree of India. The plant is present abundantly in various parts of India and even in different countries like Pakistan, Srilanka, Bangladesh etc. were conducted on rats using methanolic bark extract and was found to be non toxic. The effect of various doses of extract (25mg/ml) was studied on *invitro* rat hemi diaphragm technique .The parameters that are estimated by this method are glucose uptake and glycogen content of rat hemidiaphragm. From the *invitro* studies we can conclude that the mechanism of Antidiabetic activity of the extract is not similar to insulin as the extract did not show any effect in the *invitro* studies.

Key Words: Diabetes mellitus, Rat hemi-diaphragm, Glucose uptake, Glycogen content

Introduction

The term diabetes mellitus describes a metabolic disorder of multiple etiologies characterized by chronic hyperglycemia with disturbances of carbohydrate, fat and protein metabolism resulting from defects in insulin secretion, insulin action, or both. It is one of the major threats to human health in 21st century^[1]. Regardless of cause, it is associated with hyperglycemia. According to Diabetes Atlas published by the International Diabetes Federation (IDF), there were an estimated 40 million persons with diabetes in India in 2007 and this number is predicted to rise to almost 70 million people by 2025. The countries with the largest number of diabetic people will be India, China and USA by 2030^[2].

Since olden days, plants are used to treat many ailments. In spite of the introduction of hypoglycemic agents, diabetes and its complications continue to be a major medical problem^[3]. Synthetic hypoglycemic agents in current use for the treatment of diabetes mellitus produce serious side effects including hematological coma and disturbances of liver and kidney. WHO recommends the use of medicinal plants for the treatment of diabetes mellitus^[4]. So the search for new antidiabetic drugs and their mechanism of action continues^[5]. The present study is concerned with the antidiabetic activity of the alcoholic extract of *Saraca indica* Linn by an *invitro* rat hemi-diaphragm model.

Saraca indica Linn (*Ashoka*) is one of the most legendary and a sacred tree of India. This versatile plant is the source of various types of compounds^[6]. In the present scenario many plant are used to treat many diseases. But *Ashoka* is an ancient and reliable source of medicine^[7]. The various parts of the plant *Saraca indica* are used both internally and externally in various systems of medicine. The seed, bark and flowers of *Ashoka* are mainly used for in menorrhagia, astringent, Diabetes, biliousness, dyspepsia, ulcers and can also be used as uterine stimulant, estrogenic effects, abortifacient^[8, 9]. Many numbers of work had been done on this plant like uterine stimulant^[10], anti-ulcer^[11], anti depressant^[12], antibacterial^[13], antioxidant^[14] and larvicidal activity^[15] etc showing its pharmacological importance.

Materials and methods

Animals

Male Wister rats weighing 150-200 g were used. They were housed in standard environmental conditions (as per Institutional Animal Ethical Committee norms) and fed with standard pellet diet and water *ad libitum*.

Plant material

The plant materials of *Saraca indica*, used for the pharmacological investigation of the activity, were collected from the local areas of Kerala in the month of February. The collected barks were cleaned from dust and other materials, and then they were dried under the shade. After confirming the dryness, the barks were chopped and pulverized in an electric grinder. The powdered barks were subjected to maceration separately.

About 600 g of powdered barks were taken and soaked in methanol for four days. Stirring of the mixture was done twice daily. After the fourth day, the mixture was filtered and the marc was pressed. Then the obtained extract was distilled and then the extract was evaporated to dryness. The yield obtained after evaporation was 23.5 g. The dried bark extract of *Saraca indica* was then stored in a desiccator for further use.

Experimental Design:

Rat Hemidiaphragm Technique:

Overnight fasted albino rats of either sex weighing 150-200 g were used for the study. The rats were killed by decapitation and diaphragms were taken out quickly avoiding trauma and divided into two halves. The hemidiaphragms were then rinsed in cold tyrode solution (without glucose) to remove any blood clots and were placed in small conical flasks containing 2 ml tyrode solution with 2000 mg % glucose and incubated as follows:

A). For Glucose Uptake: -

The hemidiaphragms were incubated for 30 min at 37⁰ C in an atmosphere at 95 % Oxygen and 5 % carbon dioxide with shaking at 140 cycles/min.

Four sets of experiments were performed. The hemidiaphragms were exposed to –

1. Tyrode solution with 2000 mg % glucose only.- control
2. Tyrode solution with glucose (2000 mg %) +insulin (0.25 IU/ml).
3. Tyrode solution with glucose (2000 mg %) +leaf extract (25 mg/ml).
4. Tyrode solution with glucose (2000 mg %) + insulin (0.25 IU/ml) + leaf extract (25 mg/ml).

The hemidiaphragm was taken out and glucose content of the incubated medium is measured by GOD/POD, enzymatic method. Glucose uptake was calculated as difference between the initial and final glucose content in the incubation medium.

B). For glycogen content:-

The hemidiaphragms were incubated in Tyrode solution with glucose (2000 mg/l) in the similar way as described for glucose uptake: only the time was extended to 90 min. Following the incubation, the hemidiaphragms were rinsed for 10-15 sec in 0.9 % NaCl at 0° C to wash off external glucose and enzyme activity. It was blotted, frozen on dry ice and glycogen content of the tissue was measured by the method of Carrol et al. The glycogen content was expressed as micromoles glucose equivalent/g tissue ^[6, 16, 17]

Results and Discussion:

By comparing the readings of methanolic bark extract Group- III (3.91 ± 0.12) and group-IV (6.48 ± 0.08) with Group-I (4.47 ± 0.11) and Group-II (8.23 ± 0.15), a significant result was obtained. The glucose uptake by the tissue in the medium of insulin(standard) was highest, whereas the glucose uptake by the tissue in the extract medium, shown a reading similar to the control.

The tissues in the medium containing both insulin and extract had shown a decreased value than the insulin alone medium and more than the control medium. In the second experiment also the tissues in the insulin containing medium were shown a highest reading for glycogen content, i.e. the mechanism by which insulin acts is peripheral glucose uptake and utilization of this glucose for glycogen synthesis. The readings shown by the tissues in extract containing mediums were comparable with that of the control.

Table 1: Effect of methanolic bark extract of *Saraca indica* Linn on glucose uptake by isolated rat hemi-diaphragm

Group	Incubation Medium	Glucose uptake (mg/g per 30 min)
Group I	Tyrode solution with glucose (2000 mg/l) control group	4.47±0.11 ^b
Group II	Tyrode solution with glucose (2000 mg/l) + insulin (0.25 IU/ml)	8.23±0.15 ^a
Group III	Tyrode solution with glucose (2000 mg/l) + methanolic bark extract of <i>Saraca indica</i> Linn (25 mg/ml)	3.91±0.12 ^{a,b}
Group IV	Tyrode solution with glucose (2000 mg/l) + insulin (0.25 IU/ml) + methanolic bark extract of <i>Saraca indica</i> Linn (25 mg/ml)	6.48±0.08 ^{a,b}

Table 2: Effect of methanolic bark extract of *Saraca indica* on glycogen content by isolated rat hemi-diaphragm method

Group	Incubation Medium	Glycogen content μ moles(glucose equivalent/g tissue)
Group I	Tyrode solution with glucose (2000 mg/l) control group	14.9±1.24 ^b
Group II	Tyrode solution with glucose (2000 mg/l) + insulin (0.25 IU/ml)	27.2±2.33 ^a
Group III	Tyrode solution with glucose (2000 mg/l) + methanolic bark extract of <i>Saraca indica</i> Linn (25 mg/ml)	15.8±0.58 ^b
Group IV	Tyrode solution with glucose (2000 mg/l) + insulin (0.25 IU/ml) + methanolic bark extract of <i>Saraca indica</i> Linn (25 mg/ml)	20.4±1.15 ^{a,b}

(The values are expressed as mean \pm SEM (n =3), a – P < 0.01 when compared with the Group I, b – P < 0.01 when compared with the Group II)

From this data the extract do not act by the mechanism of glucose uptake or glycogen synthesis. So its mechanism of reduction in blood glucose is not similar to insulin. It may be due to the inhibition for the action of insulin by the extract in the medium. The mechanism of this inhibition may be that the extract binds or absorbs the insulin during the incubation period or it may be due to non-specific inhibition of different tissue enzymes as observed with sulphonylurea derivatives and sulphonamide drugs. From this we can conclude that the mechanism by which the extract acts is not the peripheral glucose uptake.

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