EFFICACY OF CEPHALANDRA INDICA MOTHER TINCTURE IN THE CONTROLLING OF BLOOD SUGAR IN THE MALE ALBINO RAT

Subasri Muthuviveganandavel¹, Muthuviveganandavel Veerappan², *Muthuraman Pandurangan³

¹Govt. Homeopathy Medical College and Hospital, Thirumangalam, Tamil Nadu, India.
²Tagore Arts College, Pondicherry, India.
³Dept. of Food Science and Nutrition, Catholic University of Daegu, South Korea

ABSTRACT

The aim of this study was to assess the effect of Cephalandra indica (MT) on male albino rat. The effect was assessed on the basis of the results of acute toxicity tests and on the comparison of the results of blood serum biochemical examinations of control and experimental groups. Group-I: sham control, group-II: Placebo control (40% ethanol), group-III: sham control with MT for 3 hrs and group-IV: sham control with MT for 6hrs were used in the study. Cephalandra indica (MT) oral administered male rats showed changes of blood serum protein, glucose, cholesterol and triglycerides content. Investigations carried out using male albino rats weighing about 125 - 150gms were administered with 1000µl/kg body weight of Cephalandra indica (MT) for group-III: Sham control with MT 3hrs and for group-IV: Sham control with MT 6hrs. At the end of the experimental period, the animals were anesthetized with diethyl ether and sacrificed by cervical decapitation. Blood was collected through cardiac puncture and stored in without EDTA containers, also used for serum biochemical analysis. Cephalandra indica (MT) effect produced decreased amount of blood serum protein, glucose and cholesterol amounts compared with controls. Changes of the serum biochemical compounds leads to positive effect in the different organs cell metabolism.

Key words: Rats; blood glucose; Cephalandra indica;protein.
INTRODUCTION

In the last few decades, there has been an exponential growth in the field of herbal medicine. It is getting popularized in developing and developed countries owing to its natural origin and lesser side effects. In olden times, vaidyas used to treat patients on individual basis, and prepare drug according to the requirement of the patient. Cephalandra indica is one of the tremendous plants of Ayurvedic system which is commonly known as kundru. It has lots of action against a various kind of diseases. It is generally used as mother tincture by the tribal people of India.

Cephalandra indica has been in use for treatment of diabetes in Ayurvedic system of medicine (Nandakarni A K1976). The other names of this plant are Coccinia indica, Momordica monadelpha. It belongs to Family: Cucurbitaceae ; Genus: Cephalandra; Specific epithet: indica – Naudin Botanical name: - Cephalandra indica Naudin (Kirtikar K.R et.al 1987). This plant grows in a wild state abundantly in Bengal and in most parts of India4. It has been described by some as the ‘Indian substitute for Insulin’ and among the medical practitioners in kolkata a strong belief exists as to its efficacy in glycosuria (Nandakarni A K1976). Another study by Chopra and Bose4 shows that it contains an enzyme with amylolytic properties, a hormone and traces of an alkaloid and it produces no reduction of sugar in the blood or urine of patients suffering from glycosuria. Ghose (1952) introduced this medicine in homoeopathy through proving and gave few case reports about its usefulness in the treatment of diabetes mellitus in mother tincture.

Diabetes mellitus (DM) refers to a group of common metabolic disorders that share the phenotype of hyperglycaemia arising as a consequence of relative or absolute deficiency of insulin secretion, resistance to insulin action, or both. The International Diabetes Federation has estimated that the total number of cases of diabetes would be about 350 million by 2030 (Kumar S et.al 2012). Hyperglycaemic conditions result in increased glycosylation. This leads further to biochemical abnormalities due to altered protein structure which over a period of time develops into diabetic complications such as nephropathy, retinopathy, neuropathy, and cardiomyopathy (ArkyR.A 1982). Pharmacotherapies for the treatment of diabetics include oral hypoglycaemic agents and insulin. These are however, not free from side effects (Bandawane D., et.al 2011).

The World Health Organization (WHO) has recommended the evaluation of traditional plants used for the treatment of diabetics as they are effective, nontoxic with fewer or no side
effects, and are considered to be excellent candidates for oral therapy (Patel K., et. al 1997). There are also several reviews on medicinal plants possessing antidiabetic activity that have been used as traditional medicines (Day C 1998; Mankil J., et. al 2006).

The present study was carried out to evaluate the antidiabetic activity of *Cephalandra indica* [mother tincture (MT) and potencies] belonging to the family of Cucurbitaceae. The medicine is used in biliousness, bilious complaints, poisonous boils, abscesses, carbuncles, blood poisoning, inflammation, and glandular swelling of the neck. *Cephalandra indica* has been acknowledged for its wound healing ability (Ghose SC 2003). It also finds use in the traditional system of Ayurveda to treat diabetes, jaundice and dysentery.

Extracts of Ivy Gourd (300 mg/kg) significantly reduced blood glucose by 47.4 and 37.1 % (P < 0.01) on 7th day and by 59.7 and 48.5 % on 14th day. A significant reduction (P < 0.01) in serum total cholesterol of 31.7 and 43.3 % and serum triglycerides of 45.5 and 39.4 % was observed on the 14th day with a single dose of the extracts of Ivy Gourd (300 mg/kg) most (Afia Akhtar, et. al 2007). The main target of the present investigation is to determine the effect of using Cephalandra indica (MT) oral administration on male albino rat blood serum biochemical.

**MATERIALS AND METHODS**

*Cephalandra indica* mother tincture (MT) was brought from Alex Homeo Pharmacy, Puducherry. Healthy male albino rats were purchased from JIPMER animal house, Puducherry, weighing (125-150 g) were selected for present study. They were kept in polypropylene cages measuring 45x77x15 cms, at temperature 25± 0.5 C, relative humidity 60 ±5% and photoperiod of 12 hrs per day. Male albino rats were grouped into four groups of six rats each: Group-I: Sham control, Group-II: Placebo control (40% ethanol), Group-III: Sham control with MT for 3hrs and Group-IV: Sham control with MT for 6hrs. Investigations carried out using male albino rats weighing about 125 - 150gms were administered with 1000µl/kg body weight of Cephalandra indica (MT) for group-III: Sham control with MT 3hrs and for group-IV: Sham control with MT 6hrs. At the end of the experimental period, the animals were anesthetized with diethyl ether and sacrificed by cervical decapitation. Blood was collected through cardiac puncture with the help of sterilized disposable syringes fitted with hypodermic needles and stored in without EDTA containers and sterilized centrifuge tubes for separation of serum. The centrifuge tubes containing blood samples were
centrifuged at 3000 rpm for 15 minutes and supernatant serum was then carefully transferred to sterilized plain glass vials and used for serum biochemical analysis.

BIOCHEMICAL PARAMETERS

1. Estimation of Blood Serum Glucose:

Blood Serum glucose was estimated by the Asatoor and King method (1954). Glucose present in the blood reacts with alkaline tartrate and phosphomolybdic acid in the reagent to yield the molybdenum blue that can be measured at 680nm in a spectrophotometer.

Reagents Required

a. Isotonic Sodium Sulphate – Copper Sulphate Solution:
   Sodium sulphate 30gm and Copper sulphate 6gm dissolved in 1 liter of distilled water was prepared.

b. Sodium Titrate: 10% Solution (10gm/dl).

c. Alkaline Tartrate Solution: In about 1 liter of distilled water 12gm of Sodium potassium tartrate, 20gm of anhydrous Sodium carbonate and 25gm of Sodium bicarbonate was added and mixed well.

d. Phosphomolybdic Acid: Commercially available product.

e. Working Standard Glucose Solution: About 25mg of glucose was dissolved in 100ml of isotonic sodium sulphate – Copper sulphate solution to obtain a standard glucose solution of 250 \( \mu l/ml \).

Procedure

To 0.1ml of blood sample, 3.8ml of Sodium sulphate – Copper sulphate isotonic solution and 0.1ml of 10% Sodium tungstate was added to get the proteins precipitated. The samples centrifuged at 3000-x g for 15 – 20 minutes for protein free solution. 0.1ml of alkaline tartrate added to 1ml of the clear supernatant and placed in a boiling water bath for 10 minutes. To the cooled solution, 3ml of phosphomolybdic acid and 3ml of water was added and mixed thoroughly. The solution was allowed to stand for 5 minutes for color development that was read against the blank at 680 nm. Blood glucose levels were expressed as mg/dl.
2. Estimation of protein
Each sample was estimated for protein content by the method of Lowry et al (1951). Reaction of a protein with alkaline copper and Folin-Ciocalteau reagents results in the blue colored solution. The intensity of the color developed was measured at 680nm and was considered proportional to the amount of protein in the sample. Bovine serum albumin (BSA) was used as the protein standard.

Reagents Required
a. 10% TCA.
b. 0.1N Sodium Hydroxide.
c. Alkaline Copper Reagent:
   Solution A: 0.5% Copper Sulphate in water.
   Solution B: 1% Sodium Potassium Tartrate in water.
   Solution C: 2% Sodium Carbonate in 0.1N Sodium Hydroxide.
   The reagent was prepared freshly, by mixing 0.5ml of solution A and 0.5ml of solution B into 49ml of solution C just prior to its use in the estimation method.
d. Folin’s Phenol reagent: Commercially available stock was diluted to 1N.
e. Standard Bovine Serum Albumin: A stock solution of 1mg/ml was prepared by dissolving 100mg of BSA in 100ml of water. 10ml of the stock was diluted to 100ml to obtain a working standard of 100μg/ml.

Procedure
To 0.1ml of tissue homogenate, 0.5ml of 10% TCA was added. The content were mixed and centrifuged. To the precipitate, 0.1ml of 0.1N NaOH was added. From this solution 0.1ml was taken, 5ml of alkaline copper reagent was added and the contents were allowed to stand at room temperature for 10 minutes. Then 0.5ml of Folin’s Phenol Reagent (1N) was added and mixed well. The blue color developed in each of the reaction tube was measured at 680nm in a spectrophotometer. A series of BSA standards (20 – 100μg), and water blank was also prepared.

3. Estimation of total Cholesterol
The total cholesterol was estimated by Zaks et al (1954) method. The serum proteins were first precipitated by treatment of the serum with Ferric chloride – Acetic acid reagent. Using a bench top clinical centrifuge the sample was centrifuged at 3000 x g
for 10 – 15 minutes to obtain a protein free supernatant. When treated with H₂SO₄ the sample gave a reddish purple color, indicating the presence of cholesterol. The intensity of the color developed was measured at 560nm.

Reagents Required
a. Ferric chloride – Acetic acid reagent: 50mg of FeCl₃ was dissolved in 100ml Acetic acid.
b. Cholesterol working standard: 40mg/ml in Ferric chloride – Acetic acid reagent.
c. Concentrated Sulphuric acid (AR).

Procedure
0.1ml serum mixed with 409ml of Ferric chloride was taken in screw capped centrifuged tube and was allowed to stand for 15 minutes. The sample was centrifuged at 3000x g for 10 minutes. 1.5ml of conc. H₂SO₄ was added to 2.5ml of the clear supernatant and incubated for about 30 minutes at room temperature for color development. The intensity of the color read against reagent blank at 560nm. A standard curve was prepared using the cholesterol standard (40mg/ml). The estimated cholesterol was expressed in mg/dl.

Statistical Analysis
Each data point represents the mean and standard deviation of three samples. Analysis was performed using a Student’s t test designed by GraphPad Software QuickCalcs. The significance and P values are indicated in data tables.

RESULT
Rat blood Serum shows in Tables -1; glucose, protein and cholesterol levels were decreased compared with control. Glucose percentage changes in Fig-1upto4.78% after 3hr and 6hr Cephalandra indica (MT) administered male rat. Similarly, protein and cholesterol percentage changes (Fig-2&3) were significantly decreased in blood serum of male rat upto 20.31% and 21.49 to 24.29% respectively compared to sham control blood serum.
Table-1: Male albino rat Serum Glucose (mg/dl), Protein (g/dl) and Cholesterol (mg/dl) amount present in Sham control, Placebo control and after 3hr & 6hr oral treatment of Cephalandra indica (MT).

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Sham Control (SC)</th>
<th>Placebo Control (PC)</th>
<th>Cephalandra indica (MT) – 3hrs</th>
<th>Cephalandra indica (MT) – 6hrs</th>
</tr>
</thead>
<tbody>
<tr>
<td>Glucose (mg/dl)</td>
<td>146.21±3.44</td>
<td>142.84±4.692</td>
<td>143.81±3.879</td>
<td>139.21±4.653</td>
</tr>
<tr>
<td>Protein (g/dl)</td>
<td>14.695±0.48</td>
<td>11.495±0.438</td>
<td>13.71±0.377</td>
<td>11.71±0.418</td>
</tr>
<tr>
<td>Cholesterol (mg/dl)</td>
<td>285.53±3.753</td>
<td>265.92±3.578</td>
<td>216.16±4.746**</td>
<td>224.16±5.442**</td>
</tr>
</tbody>
</table>

Values were represented by Mean ± SEM; Mean values were calculated by six male rats.

* = P ≤ 0.05; ** = P ≤ 0.001

Fig-1: Male albino rat Serum Glucose (mg/dl) amount present in Sham control, Placebo control and after 3hr & 6hr oral treatment of Cephalandra indica (MT).
Fig-2: Male albino rat Serum Protein (g/dl) amount present in Sham control, Placebo control and after 3hr & 6hr oral treatment of Cephalandra indica (MT).

Fig-3: Male albino rat Serum Cholesterol (mg/dl) amount present in Sham control, Placebo control and after 3hr & 6hr oral treatment of Cephalandra indica (MT).

DISCUSSION

Oral administration of Cephalandra indica (MT) into the male rat shows their decrease of blood serum glucose level leads to hypoglycemic condition followed to inhibit diabetic. The present Indian drug Cephalandra indica is one of them, which showed its usefulness in treating patients suffering from diabetes mellitus (Ghose SC 2003; Rastogi D.P, et.al 1988). Diabetes mellitus is syndrome, initially characterized by a loss of glucose homeostasis resulting from defects in insulin secretion, insulin action both resulting in impaired metabolism of glucose and other energy-yielding fuels such as lipids and protein (Florkowski
Hypoglycaemic herbal constituents reportedly have an insulin-secretagogue role in stimulating skeletal muscles, preventing neurological deficits, suppressing stress-activated protein kinase, regenerating beta-cells in the islets of Langerhans, and many phenolics compounds in them may modulate signal transduction pathways (Joseph B, 2013). Cephalandra indica mother tincture and potencies showed a significant reduction of blood glucose level, regain of body weight, and regeneration of beta-cells in the pancreas of the mother tincture-treated rats. Mother tincture-treated 3T3 cells also showed reduced uptake of glucose in comparison to normal cells (Arindam Pal et al. 2013). Similarly decreased content of cholesterol, which prevents the effect of hyperlipidemia in the blood, and safeguard from the risk factors for heart disease and obesity.

Blood serum protein content decreased due to proteolysis and transamination in the blood.

CONCLUSION
This study shows some positive role of Cephalandra indica (MT) for decreasing blood serum sugar and cholesterol level. Future controlled studies with Cephalandra indica alone vis-à-vis other conventional anti-diabetic medicines, by doing the required laboratory tests, are suggested to explore more about the hypoglycemic effect of Cephalandra indica. In summary, Cephalandra indica (MT) indicates positive effect of decreased glucose, cholesterol and protein levels in the male albino rat blood serum comparing the control groups. Our study confirmed that Cephalandra indica (MT) seems to be a practically hypoglycemic (antidiabetic effect) to rat. However, further studies are necessary, to confirm this evidence.

REFERENCES