

EFFECT OF AEGLE MARMELOS LEAF EXTRACT ON GLUCOSE UPTAKE USING ISOLATED RAT DIAPHRAGM

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Abstract

The plant extract of the aegle marmelos, a medicinal plant was used for the solely purpose to know whether is it any better or equal to the standard diabetic drug rosiglitazone by using the isolated diaphragm of the rat. Tyrode solution was prepared and accordingly extracts were divided into 7 groups with those comparing with glucose concentration of the drug after the tissue was added with standard drug and with leaf extract, leaf extract of 0.1mg and 1 mg, 5mg and 10mg was taken. Results showed that only 10mg leaf extract had show a significant uptake of the glucose which is equal to uptake of standard drug.

Keywords: Tyrode Solution, Aegle Marmelos, Standard Drug, Insulin.

INTRODUCTION

More number of research papers and reviews clearly shows that medicinal plants exhibit a variety of therapeutic properties and could provide health security to rural people primary health care. Among medicinal plants Aegle marmelos Correa (family Rutaceae) is very relevant and this plant is available in India, Bangladesh, Burma and Sri Lanka. A. marmelos has an important place in medicine. regarding pharmacology, alcoholic and aqueous extracts of the leaves had similar uses as digoxin in amplitude and contractions of the frog heart and methanolic extracts of roots inhibited the beating rate by approximately 50% of cultured mouse myocardial cells Karunanayake; Sabu and With respect to clinical applications, it should be noted that the roots are astringent, bitter and febrifuge. They are useful in diarrhea, dysentery, dyspepsia, stomachalgia cardiopalmus, seminal weakness, vomiting, intermittent fever swellings. Leaves of the A. marmelos are used as laxative expectorant, also in ophthalmic uses, inflammations, cataract, diabetes, asthmatic and antifungal complaints Also, the effect of these extracts was examined in the regulation of hyperthyroidism and for the analgesic activity in mice.

Diabetes mellitus, also referred as diabetes, is a group of metabolic diseases in which a person has high levels of blood sugar, which can be either because the body does not produce enough insulin, or because unresponsiveness of the cell towards insulin that is produced (Shoback et al., 2011). This increased blood glucose levels shows the symptoms of polyuria (frequent urination), polydipsia (increased thirst) and polyphagia (increased hunger).

A RISING GLOBAL BURDEN

Globally as of 2010, an estimated 285 million people have type 2 diabetes, making up about 90% of all diabetes cases (Shlomo Melmed et al., 2010)

Diabetes mellitus (predominantly type 2) is a major and growing health problem in almost all countries. Globally, the prevalence of diabetes in adults aged over 20 years was estimated to be 4% in 1995 and is projected to rise to 5.5% by 2025. Over the same period, the number of people effected with diabetes will rise from 135 million people to 300 million people, about 75% of whom will live in developing countries. In the western pacific region, the current number of people with diabetes is estimated to be 30 million. This will rise to at least 55millions adults by 2025. Of these 38 million will be in china and 9 million in Japan. The prevalence of diabetes exceeds 8%in 12 countries and areas of the region and in some pacific island countries it exceeds 20%. In countries where lifestyle changes began only recently (e.g. Cambodia, Vietnam) diabetes prevalence is relatively low, but there are signs that this is changing, in these countries rapid increases in prevalence can be anticipated unless urgent preventive action is taken. The numbers of people with diabetes will more than double over the next 25 years, to reach a total

of 366 million by 2030. Most of this increase to occur at a result of a 150% rise in developing countries. the fact that people migrating from rural areas to cities, particularly in growing countries. This affects the number of people with diabetics, because people living in cities in developing countries tend to be less physically active and have higher levels of overweight and obesity than people in rural areas. In fact, current situations in obesity suggests that these projections are conservative and that the growth in the prevalence of diabetes may be even higher. In developing countries it is people in the mid aged, productive years of their lives that are majorly affected by diabetes. In these countries more than half of all the people with diabetes are under 65 years old and 25% of all adults diabetes are younger than 44. in developed countries, more than half of all people with diabetes are older than 65, and only 8% of adults with diabetes are younger than 44.

The pacific island countries exhibit some of the highest recorded prevalence's of diabetes globally. Other countries in the Asian parts of the region are undergoing emerging epidemics of the disease. In 2000, 3.2 million people died from diabetes associated complications. In countries with increasing diabetic prevalence, like those in the pacific and the middle east, as many as one in four deaths in adults aged between 34 and 64 years is due to diabetes. Diabetes has become the one of the primary cause of premature illness and deaths in many countries; mainly through the highly increased risk of cardiovascular disease, cardiovascular disease is responsible for the 50%-80% of deaths in people with diabetes. Diabetes is one the primary trending cause of blindness, amputation and kidney failure. These complexities accounts for much of the financial and social burdens of diabetes. though diabetes is sometimes considered as a major condition of developed nations, the death rate from premature death among persons with diabetes is higher in developed countries than the undeveloped. The rate of premature deaths from diabetes is equal to that of HIV/AIDS, yet the problem is not recognized.

The prevalence of diabetes is growing fastly especially in the urban population in India. Since 1971-2000, a 10 fold increase has been observed (from 1.2% to 12.1%). It has remained an urban phenomenon till date and all the previous epidemiological studies have demonstared a 4 fold difference in the prevalence of diabetes between the urban and rural population. National urban diabetes survey in 2000 by a group of doctors found that Hyderabad topping the list (16.6% of its population) followed by Chennai (13.5%), Bangalore (12.4%) Kolkata (11.7%) Delhi (11.6%) and Mumbai (9.3%). The incidence in most metros and cities in India presently is 10-15%. No wonder India is the diabetic capital of the world (King et al., 1998). Diabetes mellitus is categorised into 4 categories: type 1, type 2, 3. Gestational diabetes and other specified ones". The other specified ones" are a group of a few dozen individual causes. The term "diabetes", usually refers to diabetes mellitus. The rare diseases diabetes insipidus and mellitus are similar in symptoms wise, but without any changes in the sugar metabolism (insipidus means "without taste" in Latin)

The term "type 1 diabetes" has replaced several former terms, including insulin-dependent diabetes mellitus (IDDM), childhood-onset diabetes, juvenile diabetes. and Likely, the term "type 2 diabetes" has replaced several previous terms, including adult-onset diabetes, and noninsulin-dependent diabetes mellitus (NIDDM), obesity related diabetes. other sources have stated "type 3 diabetes" as: Gestational insulin-resistant type I diabetes (or "double diabetes"), type 2 diabetes which has progressed to require insulin injection, and latent autoimmune diabetes of adults.

TYPE I DIABETES MELLITUS:

Type 1 Diabetes is the form of disease due primarily to B-cell destruction. This usually leads to type of diabetes in which insulin is required for survival. Type I Diabetes usually is characterized by the presence of anti-GAD, anti-islet cell, or anti-insulin antibodies, which reflect the autoimmune process that, have led to B-cell destruction. Individuals who have one of more of these antibodies can be sub classified as having type 1A, immune-mediated type 1 diabetes. Type 1A diabetes shows strong associations with specific haplotypes or alleles at the DQ-A and DQ-B loci of the human leukocyte antigen (HLA) complex. The rate of B-cell destroyed is quite variable, being immediate in few individuals, especially in infants and children, and slower in adults. Some have unbalanced fasting hyperglycemia that can rapidly change to severe hyperglycemia or ketoacidosis and others, particularly adults, may retain some residual B-cell function for many years and have some times been termed as having "latent autoimmune diabetes. Type 1B or idiopathic, diabetes is characterized by low insulin and c-peptide levels similar to those in Type 1A. Such patients are more vulnerable to ketoacidosis, although they have no supporting clinical evidence of autoimmune antibodies.

TYPE II DIABETES MELLITUS:

Type II diabetes is the most common form of diabetes formerly known as non-insulin dependent diabetes (NIDDM), sometimes referred to as adult-onset DM, usually begins after age 40 as a multifactorial disease that may causes improper release of insulin, malfunctioning insulin

and/or insulin resistance in peripheral tissues, either of-which may be the predominant feature. Ketoacidosis seldom occurs spontaneously but can arise with stress associated with another

illness such as infection. The pancreas plays a primary role in the metabolism of glucose by secreting the hormones insulin and

glucagon. The Islets of Langerhans secrete insulin and glucagon directly to the blood flow. Lower amount of insulin, improper structure or function of insulin or its receptors results in impaired metabolism of glucose, carbohydrates, proteins and fats, characterized by hyperglycemia and glycosuria. most frequently observed sign of diabetes is hyperglycemia and is considered the etiologic source of diabetic complications.

Aims and Objectives

Based on the previous literature numerous experimental studies have reported hypoglycemic and anti-diabetic activity of aegle marmelos leaf extract. There are no studies on the proposed mechanisms as to how this extract is producing the anti diabetic activity

- Our study was mainly intended to evaluate the mechanism by which aegle marmelos leaf extract shows the anti-diabetic activity.
- Hence, this study was planned to evaluate the in-vitro glucose uptake mechanism of aegle marmelos leaf extract produced anti-diabetic activity using isolated rat diaphragm as a skeletal muscle.

MATERIALS & METHODS

Animals

The wistar rats of either sex were used for the in-vitro glucose uptake studies. All the animals were fed with regular rat pellets and water ad libitum and maintained under standard laboratory conditions. The experimental protocol was accepted by the Institutional Animal Ethics Committee and by the Animal Regulatory Body of the Government. KVSr Siddhartha College of Pharmaceutical Sciences, Regd No: 993/a/06/CPCSEA)

FEED

Feed was purchased from vet care international ltd. for experimental laboratory animals. Our feed was entirely different from those of the popular feed (Dry Pellets) available in the market. The Key advantages of nutrilib feed are better nutrient profile, better stability, no wastage of feed, less microbial load etc.

PLANT EXTRACTION PROCEDURE:

Preparation of 50% ethanol extract of Aegle marmelos

Leaves (500 g) were washed with distilled water (dH2O) to remove dirt and soil, and were properly dried in shade for 4-7 days then dried in tray drier, maintained at 40°C. After drying, the plant materials were milled to powder and passed through the sieve (mesh size 40).The powdered materials were mixed in 50% ethanol solution (EtoH) for two days. The extract was separated by filtering and concentrated on rota vapour and then dried in the lyophilizer under reduced pressure to obtain solid residue (Root yield- 3.2% and Leaves yield- 1.6% w/w).

Glucose uptake measurement

Six best microtitre plates were selected for this study with each having a capacity of 5 ml (n = 4). Plates were divided into following groups. For each group, three tissues were taken from the respective three animals after being sacrificed for the estimation of in-vitro glucose uptake on isolated rat diaphragm. Experimental was design was given as follows.

Experimental Design:

| | |
|---------|---|
| Group 1 | 2 ml of tyrode solution with 2G of glucose/L |
| Group 2 | 2 ml of tyrode solution with 2G of glucose/L and regular insulin 5µL containing 0.2U of insulin |
| Group 3 | 2 ml of tyrode solution with 2G of glucose/L, regular insulin 5µL containing 0.20 of insulin, standard drug rosiglitazone |

| | |
|---------|--|
| Group 4 | 2 ml of tyrode solution with 2G of glucose/L, regular insulin 5 μ L containing 0.2U of insulin, Aegle marmelos leaf extract 0.1 mg |
| Group 5 | 2 ml of tyrode solution with 2G of glucose/L, regular insulin 5 μ L containing 0.20 of insulin, Aegle marmelos leaf extract 1mg |
| Group 6 | 2 ml of tyrode solution with 2G of glucose/L, regular insulin 5 μ L containing 0.2U of insulin, Aegle marmelos leaf extract 5mg |
| Group 7 | 2 ml of tyrode solution with 2G of glucose/L, regular insulin 5 μ l containing 0.2U of insulin, Aegle marmelos leaf extract 10mg |

Wistar rats of both sexes were maintained on a standard pellet diet, water ad libitum, and fasted all the night. The animals were killed by excess anesthesia and diaphragms were taken out swiftly avoiding trauma and divided into two halves. The hemi-diaphragms were then rinsed in cold Tyrode solution (without glucose) to remove any blood clots and about 200 mg tissues were transferred to the respective wells. The plates were covered close with the lids and incubated for 45 min at 21°C with shaking at 60 cycles per min (Prashantha Kumar et al., 2010).

Following the incubation, the blood glucose levels were measured by strip method by taking directly drop of blood on strip from the rat tail vein (Subramanian et al, 1998) and inserted strip immediately into calibrated glucometer and blood glucose level reading were recorded by

Glucometer: ACCU-CHECK

Glucose uptake =

(Glucose Conc. of tyrode before taking tissue) - (Glucose Conc. of tyrode after taking tissue

in the presence or absence of drugs)

Glucose concentration was expressed as mg/dl

Glucose uptake was expressed as mg/200mg tissue/45min

TYRODE SOLUTION COMPOSITION

| Substance | Amount in g/L |
|----------------------------------|---------------|
| NaCl | 8.00 |
| KCl | 0.20 |
| CaCl ₂ | 0.20 |
| MgCl ₂ | 0.10 |
| NaH ₂ PO ₄ | 0.05 |
| NaHCO ₃ | 1.00 |
| Glucose | 2.00 |
| pH6.5 | |

DISSOLUTION OF PLANT EXTRACTS:

Dried powder of Aegle marmelos leaf extract was dissolved in 30% DMSO + 10% Tween 80 + 60% Tyrode solution

Standard drug, Rosiglitazone dissolved in 90% methanol

RESULTS

Table1: effect of A.marmelos extract on glucose reuptake by isolated rat hemi diaphragm in vitro assay

| Groups | Before adding tissue (Glucose conc.mg/dl) | After adding tissue (Glucose conc.mg/dl) |
|--|--|---|
| Group 1 Control solution | 118±3 | 116±4 |
| Group 2 Control+ 5µL regular insulin containing 2U | 117±4 | 101±3 |
| Group 3 Control+ insulin+ standard drug | 119±5 | 84±3 |
| Group 4 Control+ insulin+ A.m leaf extract 0.1mg | 115±4 | 99±3 |
| Group 5 Control+ insulin+ A.m leaf extract 1mg | 102±2 | 94±2 |
| Group 6 Control+ insulin+ A.m leaf extract 5mg | 112±3 | 98±2 |
| Group 7 Control+ insulin+ A.m leaf extract 10mg | 109±2 | 84±3 |

DISCUSSION

Aegle marmelos would act like insulin in the restoration of blood sugar. Recently another study also demonstrated the hypoglycemic activities of aqueous extracts of fruits of this plant (Kamalakkannan, 2005) In addition, another study reported that aqueous seed extract of Aegle marmelos possess anti-diabetic and hypolipidemic effects in diabetic rats (Kesari et al., 2006). One possible mechanism of anti-hyperglycemic activity is majorly due to glucose uptake into muscles and this idea was supported by recent study in which Aegle marmelos Correa root increases the In vitro glucose uptake by isolated rat hemi-diaphragm study (Subban et al., 2009). In the present study, results demonstrated that the aegle marmelos leaf extract at a dose of 10mg dose has shown significant in-vitro glucose uptake activity compared to activities of 0.1, 1 and 5 mg. Though glucose uptake is dose dependently increasing for the doses 1, 5 and 10mg, only significant effect was observed with the 10mg dose.

CONCLUSION

In-vitro glucose uptake was estimated using isolated rat diaphragm. Here, standard drug, Rosiglitazone 2mg has shown significant glucose uptake in our glucose uptake model. Since standard drug is working well, our in-vitro model of glucose uptake was considered as a valid model to evaluate the test extracts with various doses.

Four doses of plant extracts (0.1, 1, 5, and 10) were chosen in to evaluate the glucose uptake in this particular study. Of the four doses tested, aegle marmelos leaf extract 10mg has shown significant glucose uptake whereas 0.1, 1 and 5mg have not shown significant glucose uptake. Though aegle marmelos leaf extract 10mg has shown significant glucose uptake but it is not on par with efficacy of standard drug, Rosiglitazone. Of course, Plant extracts are safer drugs than the glitazones hence, these plant extracts can be preferable for the clinical use. Aegle marmelos leaf extracts are already being used in the traditional system of medicine and numerous studies authenticated anti-diabetic activity of this plant extract. But, no evidence is available on the mechanism of this plant extract as to how it is showing anti-diabetic activity. In this context, our study is providing a clue for further research in elucidating the cellular mechanisms.

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