Evaluation and comparison of anti-diabetic activity of hydroalcoholic extracts of fresh and dry leaves of *Psidium guajava* in type-II diabetes mellitus

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**ABSTRACT**

*Psidium guajava* is one of the most widely used plant as herbal remedy for the treatment of Diabetes mellitus. In the present study the hydroalcoholic extracts of the fresh and dry leaves of this plant are therapeutically evaluated and compared for anti-hyperglycemic potential against Alloxan induced Diabetes in Rats. The animals were administered with doses of 500mg/kg body weight of extract orally to Alloxan induced rats continuously for 30days, caused significant reduction in the fasting serum blood glucose levels. Among the two extracts fresh leaf extract showed significant anti hyperglycemic activity than the dry leaf extract which nearly produced equal reduction in serum blood glucose levels to that of standard glibenclamide 10mg/kg.

**Key words:** *Psidium guajava*, Alloxan, Hydro alcoholic extracts, anti-hyperglycemic, Glibenclamide.

**INTRODUCTION**

Type-II Diabetes Mellitus is a non-autoimmune, complex, heterogeneous and polygenic metabolic disease condition in which the body fails to produce enough insulin, characterized by abnormal glucose homeostasis. Its pathogenesis appears to involve complex interactions between genetic and environmental factors Type-II Diabetes Mellitus occurs when impaired insulin effectiveness (insulin resistance) is accompanied by the failure to produce sufficient insulin.

It has been estimated that the global burden of Type II diabetes mellitus for 2010 would be 285 million people (2010) which is projected to increase to 438 million in 2030, a 65 % increase. Similarly, for India this increase is estimated to be 58%, from 51 million people in 2010 to 87 million in 2030 [1]. The problem of diabetes is particularly relevant to India, according to the recent Diabetes Atlas produced by the International Diabetes Federation (IDF), India is home to the largest number of people with diabetes in the world [2], and so India is referred as Diabetic capital of the world.

Plants have always been an exemplary source of drugs. Herbal industry in India uses about 8000 medicinal plants and the annual turnover of the Indian herbal medicinal industry is more lucrative [3] and nearly about 800 plants are known to possess antidiabetic potential [4], many of the currently available drugs have been derived directly or indirectly from them.

According to “the wealth of India” of council of scientific and industrial research, New Delhi in 1956 (it is a dictionary of Indian raw materials and industrial products) *Psidium guajava* belongs to the family Myrtaceae. It is a tropical shrub grows in regions up to 1, 500 m above mean sea-level. Guava is cultivated on varied types of soils- heavy clay to very light sandy soils. It tolerates a soil pH of 4.5- 8.2. Mainly grown in tropical and subtropical regions. The leaves and bark are claimed to have medicinal properties [5]. *Psidium guajava* Linn. (Guava) is used not only as food but also as folk medicine in subtropical areas around the world because of its pharmacologic activities. In particular, the leaf extract of guava has traditionally been used for the treatment of diabetes in East Asia and other countries. It is generally used in the treatment of diarrhoea, jaundice, dysentery, conjunctivitis, cough, Rheumatism [6, 7] whole fruit is found to possess good anti-oxidant activity [8, 9, 10].

However the hydro alcoholic extracts of fresh and dry leaves of *Psidium guajava* was not comparatively studied and evaluated so far for its anti-diabetic properties, and the LD50 of leaf...
extracts of *Psidium guajava* is as high as 2000mg/kg b.w. in rats and mice with no mortality or adverse effects indicating its safety \[^{[11]}\]. Hence in the present study the anti-diabetic property of the fresh and dry leaf extracts of the plant are evaluated and compared.

**MATERIALS AND METHODS**

**Chemicals:** Alloxan monohydrate and Glucose-oxidase peroxidase kit was purchased from sigma-Aldrich chemicals, Mumbai.

**Collection of plant material:** fresh matured leaves of *Psidium guajava* were harvested from the coastal areas of Visakhapatnam. The leaves were washed with tap Water, rinsed with distilled water and shade dried to remove traces of water and dust.

**Preparation of hydro alcoholic leaf extracts:** Fresh leaf and dry leaf extracts are prepared by method of continuous soxhlet extraction. Freshly collected leaves were dried under shade and the dried material was milled to obtain a coarse powder. The coarse powder (50gm) was packed in a soxhlet apparatus and macerated with little amount of alcohol, later subjected to continuous extraction with a mixture of 105ml of ethanol and 45ml of distilled water. In the same way fresh leaf extract was also prepared.

The liquid extract was collected and evaporated by placing the china dish containing liquid extract on a hot plate until a soft mass was obtained. The mass obtained was weighed in both the cases. The extract was thoroughly air dried to remove all the traces of solvent.

**Preparation of test samples:** The dried and fresh extracts were suspended in 2% gum acacia suspension in distilled water prior to oral administration to animals was used as the reference drug. Animals in the control group received only the vehicle.

**Animals:** Wistar rats (150-200g) were selected for the study and maintained at controlled temperature of 19-25°C with 12hr light/dark cycle and fed with a standard diet and water ad libitum. The experiments were conducted according to the institutional Animal Ethics committee regulations.

**Anti-diabetic activity:** Rats were divided into five groups, each group containing five animals, Group-I served as Non-diabetic control, which received 2% of Gum acacia in saline. Groups II-IV are fasted overnight and administered with alloxan 120mg/kg, (i.p) in saline, \[^{[12]}\] and the animals which produced significant increased blood glucose levels of 180mg/dl and above are included in the study. Group-II served as Diabetic control received 2% gum acacia in saline, Group-III served as reference standard which received Glibenclamide 10mg/kg orally dissolved in saline, Groups IV and V received dry leaf extract(500mg/kg) and fresh leaf extract(500mg/kg) of *Psidium guajava* respectively. All these treatments were given orally for 30days and on the final day the blood samples were collected by retro-orbital plexus puncture method, and the serum glucose levels are estimated by glucose-oxidase-peroxidase method.

**Statistical analysis:** The results were expressed as Mean±S.E.M, and the statistical evaluation carried out by t-test, and the probability of 0.05 was chosen as level of significance.

**RESULTS**

From the data obtained Alloxan induced diabetic rats showed a significant reduction in blood glucose levels (*p<0.05*) with both dry and fresh leaf extracts, alloxan produced rapid induction of diabetes, the standard glibenclamide 10mg/kg produced a significant reduction of blood glucose levels. The hydro alcoholic extract of fresh leaves of *Psidium guajava* produced a very significant reduction in blood glucose levels similar to the Glibenclamide, the results obtained were represented in the table.

**Table: Comparison of anti-diabetic activity of hydro alcoholic extracts of fresh and dry leaves of *Psidium guajava***

<table>
<thead>
<tr>
<th>Test sample</th>
<th>Mean blood glucose levels ± S.E.M(mg/dl)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Before</td>
</tr>
<tr>
<td>Group-I(control)</td>
<td>93.69±1.36</td>
</tr>
<tr>
<td>Group-II(Diabetic control)</td>
<td>94.24±1.32</td>
</tr>
<tr>
<td>Group-III(standard)</td>
<td>186.14±3.83</td>
</tr>
<tr>
<td>Group-IV(dry leaf extract)</td>
<td>190.02±1.89</td>
</tr>
<tr>
<td>Group-V(fresh leaf extract)</td>
<td>188.49±1.23</td>
</tr>
</tbody>
</table>

n=5, p value<0.05 level of significance
DISCUSSION

Several medicinal plants are widely used for the treatment of diabetes mellitus in the traditional medicine of many countries; the present work was carried out to know whether the plant has antidiabetic effects. Studies revealed that the plant contains good anti-diabetic potential, with increase in the body weights of the animals. As per the literature review the plant also contains anti-oxidant properties [13]. So free radical scavenging property exists for the compound which may play a key role in minimising the microvascular and macrovascular complications of diabetes mellitus.

Fig: comparison of anti-diabetic activity of hydro alcoholic extracts of fresh and dry leaves of *Psidium guajava*

**Mean blood glucose levels (mg/dl)**

<table>
<thead>
<tr>
<th>Group</th>
<th>Mean Blood Glucose Levels (mg/dl)</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>Before 93.69±4.46</td>
</tr>
<tr>
<td>II</td>
<td>After 94.24±0.98</td>
</tr>
<tr>
<td>III</td>
<td>Before 189.26±2.44</td>
</tr>
<tr>
<td>IV</td>
<td>After 186.14±0.95</td>
</tr>
<tr>
<td>V</td>
<td>Before 190.02±0.99</td>
</tr>
<tr>
<td></td>
<td>After 188.49±0.96</td>
</tr>
</tbody>
</table>

6. Anil Kumar , Importance for Life ‘Psidium guava; *International Journal of Research in Pharmaceutical and Biomedical Sciences*, 2012; 3(1):137-143  