RESEARCH ARTICLE

Evaluation of *Garcinia indica* Whole Fruit Extracts For Hypoglycemc Potential in Streptozotocin Induced Hyperglycemic Rats.

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ABSTRACT:

*Garcinia indica* (GI) whole fruit extracts i.e. aqueous, methanol and chloroform were evaluated for its hypoglycemic activity in oral glucose tolerance in glucose loaded euglycemic rats and in streptozotocin (STZ) 50mg/kg induced hyperglycemic rats. Acute treatment of aqueous extract (400mg/kg) markedly improved oral glucose tolerance in glucose (3g/kg) loaded euglycemic rats. Treatment of aqueous extract (400mg/kg) in STZ induced hyperglycemic rats showed significant (p<0.0001) reduction in the fasting blood glucose levels both in acute and chronic study, indicating its antihyperglycemic activity. Methanol and chloroform extracts treatment do not showed significant hypoglycemic activity. The result of the present study indicates aqueous extract of GI whole fruit posses significant hypoglycemic activity in STZ induced hyperglycemic rats.

KEYWORDS: Hyperglycemia, *Garcinia indica* fruit, hypoglycemic activity, Streptozotocin, OGTT

INTRODUCTION:

Herbal drugs have served as a major source of medicine for the prevention and treatment of many diseases including diabetes mellitus¹. Many herbal plant spices were showed hypoglycemic properties, including common plants such as pumpkins, wheat, celery, bitter melon².

Diabetes mellitus is a clinically syndrome characterized by an inappropriate hyperglycemia caused by lack of insulin or insulin resistance or both at the cellular level (3). Recent analysis indicates the human population worldwide appears to be in the midst of epidemics of diabetes. Despite the great stride that have been made in the understanding and management of diabetes, the disease and disease related complications are increasing unabated⁴.

Ayurveda and other traditional medicine systems describe number of plants used as herbal drugs for the treatment of diabetes mellitus. The active principles present in herbal drugs have been reported to possess hypoglycemic potentials acting through different pathways⁵. It is also reported that plants useful in diabetes mellitus possess strong antioxidant/free radical scavenging properties⁶.

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The aqueous extract was prepared by macerating 100 gm of defatted powder with chloroform water IP for seven days with occasional shaking at room temperature, filtered and concentrated on rotary evaporator and dried in desicator over sodium sulphite. The yield obtained was 34.80 % w/w.

Chloroform and methanol extracts were prepared with 100 gm of defatted powder in soxhlet extractor and extracted first with chloroform (40-60ºC) and then with methanol 95% v/v at 60ºC. Appearance of colourless solvent in the siphon tube was considered for termination of extraction process. The yield obtained was 19.44% and 30.48% w/w respectively. All the extracts were stored in refrigerator at 4ºC until further use for experimental study.

**Phytochemical screening:** A preliminary phytochemical analysis was carried out of all the three extracts (aqueous, methanol and Chloroform) employing the standard phytochemical procedures to reveal the presence of various phytoconstituents.

**Animals:** Healthy Wistar rats (180-200g) of either sex, obtained from Venkatesh enterprises Bangalore, were used. They were maintained on standard animal pellet diet (Amrut animal feed, Sangli, Maharashtra) and water *ad libitum*. The present study was dully approved by IAEC bearing Reg. NO 627/02/a/CPCSEA JN Medical College Belgaum.

**Acute oral toxicity study:** Acute oral toxicity study was carried out by using Wistar rats by “fixed dose” method of OECD guideline NO. 420 and a starting dose of 2000 mg/kg body weight was adopted. There were no toxic effects or mortality observed up to 14th days with all the three extracts.

**Evaluation of hypoglycemic activity:** GI whole fruit extracts i.e. aqueous, methanol and chloroform were screened to find out hypoglycemic effect by OGTT method and in STZ induced hyperglycemic rats (acute and chronic model).

i) **Oral glucose tolerance test (OGTT)**: The effect of all three extracts were evaluated in glucose loaded (3g/kg) normal rats. There were total eight groups consisting each of six normal rats. Group I served as control rats given normal saline (2ml/kg p.o). Group II served as standard treated with glibenclamide (0.9mg/kg p.o), while groups (III to V) served as test groups treated at a dose 200mg/kg, where as groups (VI to VIII) treated at a dose of 400mg/kg i.e aqueous, methanol and chloroform extracts respectively.

After fasting for 12-16 hrs all animals in each group were orally administered vehicles, glibenclamide or the different extracts 30 min prior to oral glucose load (3g/kg) respectively. The blood glucose levels were estimated from each group before (0 min) extracts administered and at 30, 60, 90, 120 and 180 min after glucose challenge.

**Animal groupings:**
- **Group I:** Control [normal saline 2ml/kg]
- **Group II:** Standard [ glibenclamide( 0.9mg/kg) + glucose 3g/kg]
- **Group III:** Test 1 [aqueous extract (200mg/kg)+ glucose 3 g/kg]
- **Group IV:** Test 2. [ methanol extract ( 200mg/kg) + glucose 3 g/kg]
- **Group V:** Test 3 [chloroform extract (200mg/kg) + glucose 3 g/kg]
- **Group VI:** Test 1[aqueous extract (400mg/kg) + glucose 3 g/kg]
- **Group VII:** Test 2 [ methanol extract ( 400mg/kg) + glucose 3 g/kg]
- **Group VIII:** Test 3 [chloroform extract (400mg/kg) + glucose 3 g/kg].

ii) **STZ induced hyperglycemic animals.** At the end of one-week acclimatization period, freshly prepared STZ at a dose of 50 mg/kg was injected intraperitonially as a single dose. Blood samples were obtained from rat tail vein on 3rd and 7th day and blood glucose was estimated by using glucometer. Rats with blood glucose levels of 200 mg/dl or more on both days were categorized as a hyperglycemic.

**Hypoglycemic test:**

a) **Acute study design:** Animals are divided in to eight groups with six in each.
- **Group I.** Hyperglycemic (STZ induced 50mg/kg)
- **Group II.** Hyperglycemic + glibenclamide (0.9mg/kg)
- **Group III.** Hyperglycemic+ aqueous extract (200mg/kg)
- **Group IV.** Hyperglycemic + methanol extract (200mg/kg)
- **Group V.** Hyperglycemic+ chloroform extract (200mg/kg)
- **Group VI.** Hyperglycemic+ aqueous extract (400mg/kg)
- **Group VII.** Hyperglycemic + methanol extract (400mg/kg)
- **Group VIII.** Hyperglycemic + chloroform extract (400mg/kg)

All STZ induced hyperglycemic rats were fasted for 12-16 hours before they were tested for the blood glucose level. A basal glucose level (BGL) was recorded on day of experiment prior to the extract administration. Later the animals in each group were orally administered with vehicle, glibenclamide (0.9 mg) or the different extracts (200 and 400mg/kg p.o single dose). The blood glucose levels were measured at 30, 60, 90, 120 and 180 minutes by using the glucometer.

b) **Chronic study design:**
GI fruit extract showing significant hypoglycemic activity in acute study was only selected for chronic study i.e. for 30 days. The same animals from the acute study were continued for chronic study and treatment given was as follows:
- **Group I:** Normal euglycemic (normal saline 2 ml/kg)
- **Group II:** STZ induced hyperglycemic rats. (Normal saline 2ml/kg)
- **Group III:** Hyperglycemic + glibenclamide (0.9 mg/kg p.o single dose)
Group IV: Hyperglycemic + aqueous extract treated (400 mg/kg p.o twice daily)

**Drugs and chemicals:** Drugs and chemical used includes streptozotocin (Himedia, Mumbai), glibenclamide (Inga Laboratories Mumbai), Glucometer and glucostrips (Sugar check, Wokhardt, Mumbai).

**Statistical analysis:** Data was expressed as Mean ± SEM and was analyzed by two way ANOVA followed by Bonferroni post tests. *P*<0.05 was considered statistically significant.

**Results**

**Acute toxicity:** All three extracts at a dose 2000 mg/kg body wt. showed no toxic effects or mortality up to 14 days. The LD<sub>50</sub> cut off value of the extracts was 2000 mg/kg body wt.

**Selection of the dose** The LD<sub>50</sub> cut off value was found to be 2000 mg/kg body wt. For the assessment of hypoglycemic activity two doses were selected i.e. first dose 1/10<sup>th</sup> of the LD<sub>50</sub> cut off value and second dose twice that of one tenth dose i.e. (200 and 400mg/kg respectively).

**Phytochemical screening:** The preliminary qualitative phytochemical screening of GI whole fruit extracts showed the presence of carbohydrates, flavonoids, alkaloids, steroids, tannins, saponins, oxalic acid, citric acid and ascorbic acid in aqueous extract. Carbohydrates, flavonoids, steroids, tannins, saponins and citric acid in methanol extract and carbohydrate, tannins, oxalic acid and citric acid in chloroform extract.

**Oral glucose tolerance test:** At a dose of 200 mg/kg body wt of aqueous, methanol and chloroform extracts exhibited insignificant fall in a BGL when compared with control group (Fig.1). Acute administration of aqueous extract at dose 400 mg/kg in normal rats showed significant improvement in oral glucose tolerance following oral glucose load as shown (Fig. 2) when compared with control group. No significant fall in BGL observed in methanol and chloroform extracts treated groups.

**Acute hypoglycemic activity:** At a dose 200mg/kg aqueous, methanol and chloroform extracts showed insignificant decreased in BGL in STZ induced hyperglycemic rats when compared with untreated hyperglycemic rats (Table 1). 400 mg/kg dose of aqueous extract showed significantly (*P*<0.001) decreased the BGL between 30 to 120 minutes. Whereas no significant decrease was observed in methanol and chloroform extract treated groups when compared with untreated hyperglycemic rats (Table 1). As aqueous extract at dose 400 mg/kg body wt. showed significantly decreased BGL with duration of action between 30 to 120 minute (Table 2), this dose was selected for chronic hypoglycemic activity and was administered twice daily for 30 days.

**Chronic hypoglycemic activity:** Administration of GI aqueous fruit extract at a of dose 400 mg/kg twice daily in STZ induced hyperglycemic rats for 30 days significantly (*P*<0.001) decreased BGL when compared with untreated hyperglycemic rats.

**Pancreatic histology:** The histopathological studies of pancreas revealed severe congestion with severe decrease in number of islets of Langherhans and beta cells with fibrosis and inflammatory cell infiltration into the islets of Langherhans in STZ induced hyperglycemic rats. While the aqueous extract of GI whole fruit at a dose of 400 mg/kg showed mild congestion with mild decrease in number of islets of Langherhans with normal beta cell population indicating significant amount of recovery. Glibenclamide treatment showed moderate congestion with moderate decrease in number of islets of langherhans and beta cells and mild lymphocytic infiltration (figures are not included).

**DISCUSSION:**

The present study was planned to evaluate hypoglycemic potential of whole fruit of GI extracts i.e aqueous, methanol and chloroform in OGTT and STZ induced hyperglycemic animals. The acute administration of aqueous extract at 400 mg/kg significantly improved oral glucose tolerance in glucose loaded normal rats indicating its antihyperglycemic activity, further acute and chronic treatment in STZ induced hyperglycemic rats also showed significant antihyperglycemic action.
Table 1. Effect of acute treatment of various extract of GI whole fruit extracts at dose 200 and 400 mg/kg body wt. on STZ induced hyperglycemic rats.

<table>
<thead>
<tr>
<th>Groups</th>
<th>Fasting blood glucose concentration (mg/dl)</th>
<th>0 min</th>
<th>30 min</th>
<th>60 min</th>
<th>90 min</th>
<th>120 min</th>
<th>180 min</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group I</td>
<td>294.00±4.99</td>
<td>296.30±5.16</td>
<td>297.30±4.78</td>
<td>298.80±4.77</td>
<td>301.20±5.53</td>
<td>303.70±5.39</td>
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<tr>
<td>Group II</td>
<td>307.20±4.99</td>
<td>286.70±4.01</td>
<td>269.80±4.05**</td>
<td>256.80±4.05***</td>
<td>245.30±6.95***</td>
<td>239.80±5.26***</td>
<td></td>
</tr>
<tr>
<td>Group III</td>
<td>292.70±4.04</td>
<td>405.20±8.75</td>
<td>350.70±9.15</td>
<td>319.80±11.15</td>
<td>312.70±9.08</td>
<td>321.00±5.58</td>
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</tr>
<tr>
<td>Group IV</td>
<td>290.00±5.36</td>
<td>402.30±11.22</td>
<td>436.00±13.93</td>
<td>421.80±20.43</td>
<td>400.30±17.93</td>
<td>384.80±8.20</td>
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<tr>
<td>Group V</td>
<td>313.50±3.25</td>
<td>399.70±4.32</td>
<td>420.20±4.87</td>
<td>422.50±8.22</td>
<td>406.70±7.12</td>
<td>395.30±5.38</td>
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<tr>
<td>Group VI</td>
<td>301.20±5.99</td>
<td>362.00±12.10</td>
<td>377.50±6.27</td>
<td>356.30±6.65</td>
<td>325.70±3.72</td>
<td>335.20±4.56</td>
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</tr>
<tr>
<td>Group VII</td>
<td>299.7±6.04</td>
<td>348.30±9.35</td>
<td>392.70±3.52</td>
<td>409.70±4.25</td>
<td>395.70±2.33</td>
<td>381.50±4.19</td>
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<tr>
<td>Group VIII</td>
<td>282.30±2.74</td>
<td>336.50±7.15</td>
<td>379.50±4.41</td>
<td>402.00±3.14</td>
<td>398.20±4.37</td>
<td>391.00±2.14</td>
<td></td>
</tr>
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</table>

*P<0.05 when compared with group I. **P<0.01 when compared with Group I. ***P<0.001 when compared with group I.
4 P<0.05 when compared with group VII. **P<0.01 when compared with group VII.

Whereas no such significant effect were seen with methanol and chloroform extracts. Phytoconstituents such as flavonoids, tannins, polyphenols, carbohydrates, saponins and Vit.C have shown hypoglycemic activity in animal studies. The preliminary qualitative phytochemical analysis of aqueous extract showed presence of carbohydrate, steroids, flavonoids, alkaloids tannins, citric acid and Vit.C. The hypoglycemic activity of GI fruit could be due to the presence of these active phytoconstituents.

STZ induction causes severe selective destruction of the beta cells of the islets of langerhans through oxidative stress induced pathway. The histological examination in the present study showed, STZ (50mg/kg i.p) single dose causes severe destruction of the beta cells. Aqueous extract of GI whole fruit treatment demonstrated significant recovery when compared with untreated hyperglycemic rats. This could be sign of regeneration of beta cell of islets of langerhans through oxidative stress and mechanism of action for hypoglycemic activity.

**CONCLUSION:**

In conclusion aqueous extract of GI whole fruit at a dose 400mg/kg twice daily showed significant (P<0.0001) hypoglycemic activity in STZ induced hyperglycemic rats. Further study is required to trace out the active constituents and mechanism of action for hypoglycemic activity.

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Abbreviations used: GI =Garcinia indica; STZ =streptozotocin; BGL = blood glucose levels

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