Amelioration of experimental diabetic neuropathy and gastropathy in rats following oral administration of plant (Eugenia jambolana, Mucuna pruriens and Tinospora cordifolia) extracts

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Extract of M. charantia (200mg/kg), E. jambolana (200mg/kg), M. pruriens (200mg/kg) and T. cordifolia (400mg/kg) was administered for 50 days in STZ induced diabetic mice, the plasma glucose concentration was reduced by 24.4, 20.84, 7.45 and 9.07 % respectively. Tail flick latency (TFL) and gastric transit percentage were significantly higher in diabetic controls versus normal controls. M. charantia and E. jambolana modified it favorably while M. pruriens and T. cordifolia did not exert any favorable change.

Diabetic neuropathy is the complication of diabetes mellitus and occurs in almost 50% of diabetic patients. Patients with autonomic neuropathy are characterized by maladaptation to everyday stress situations like exercise and hypoglycemia which predispose them to a higher risk of death. Gastrointestinal symptoms are also responsible for substantial morbidity and approximately 30 to 50 % of patients with long-standing diabetes mellitus have delayed gastric emptying time and up to 60% have severe intestinal symptoms like nausea, vomiting, diarrhoea and constipation. Presently, other than symptomatic control no effective treatment is available though metabolic control has been shown to strongly influence the development of diabetic neuropathy and gastroparesis. In the recent years, popularity of complementary medicine has increased as shown by surveys conducted in Australia and US, which indicate that almost 48.5 and 34 % respondents had used at least one form of unconventional therapy including herbal medicine. Dietary measures and traditional plant therapies prescribed by Ayurveda and other indigenous systems of medicine have been used commonly in India. In the present study, 4 plants namely Monordica charantia; Eugenia jambolana; Mucuna pruriens; and Tinospora cordifolia with previously confirmed anti-hyperglycemic activity were selected for the present study.

Anti-hyperglycemic activity of M. charantia, E. jambolana, M. pruriens and T. cordifolia has been reported earlier but none of these studies have revealed the effect of plant extracts on development of diabetic complications such as neuropathy and gastropathy. In our previous studies, M. charantia and E. jambolana have shown higher anti-hyperglycemic effect as compared to T. cordifolia and M. pruriens in mild (plasma sugar > 180 mg/dl, duration 21 days), moderate (plasma sugar > 280 mg/dl, duration 120 days) and severe (plasma sugar > 400 mg/dl, duration 60 days) diabetic rats. In addition, M. charantia and E. jambolana also partially restored altered hepatic and skeletal muscle glycogen content and hepatic glucokinase, hexokinase, glucose-6-phosphate and phosphofructokinase levels. Moreover, E. jambolana and M. charantia also attenuated certain parameters in STZ induced diabetic nephropathy and dietary fructose induced insulin resistance in normal rats.

Materials and Methods

Extract of plant parts—Fresh green fruit of Monordica charantia and dried kernels of ripen Eugenia jambolana were collected locally. Cuticle of M. charantia fruit was peeled off and macerated in an electric mixer (Electro Com, New Delhi), the kernels of E. jambolana were grounded in an electric grinder. The macerated pulp of M. charantia and the E. jambolana powder were then soaked separately in equal amount of water and stirred intermittently and then left overnight. This pulp was then filtered.

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through a coarse sieve and the filtrate was dried at reduced temperature. Respective yield of *M. charantia* and *E. jambolana* was 80.4 g/kg of fruit and 73 g/kg of kernel. To increase the shelf life and uniformity, the extracts were completely lyophilized by continuous freeze drying operation of 54 hr. (Christ freeze dryer, alpha 14, Germany), yielding 64 and 51 g/100g of *M. charantia* and *E. jambolana* extract respectively.

Alcoholic and aqueous extracts of *T. cordifolia* and *M. pruriens* were received as gift from Brawn Pharmaceuticals LTD, Faridabad (India). All the extracts were suspended in 1% carboxymethylcellulose (Central Drug House, New Delhi) and given orally.

**Sample collection**—Blood was collected retro-orbitally from the inner canthus of the eye under light ether anesthesia using capillary tubes (Micro Hematocrit Capillarys, Mucaps). Blood was collected in fresh vials containing sodium fluoride and sodium oxalate as anti-coagulant/anti-glycolytic agents and plasma was separated in a T8 electric centrifuge (Remi Udyog, New Delhi) at 2000 rpm for 2 min.

**Preparation of diabetic animals**—Male albino mice (20-35g) were fasted for overnight and given a bolus injection of STZ (150 mg/kg; ip) dissolved in citrate buffer (3mM; pH 4.5). After 10 days, serum glucose was estimated using commercially available Autopak® kit, Bayer Diagnostics, Baroda. Mice exhibiting plasma glucose levels >300 mg/dl were used for further study.

**Experimental design**—Albino mice (30-50g) of both sexes (8-10 weeks old) were obtained from the Institute and maintained on standard chow diet during the experiment. The animals were randomized in the following groups with a acclimatization period of 2-3 days before initiation of experiment. Mice were divided into 6 groups having 8 mice in each group. In group I, mice received saline daily and served as normal control. In group II to VI, mice received a single intra peritoneal injection of 150 mg/kg STZ. In group II, mice received saline daily and served as a diabetic control. Group III received 200 mg of lyophilized powder of *M. charantia*, group IV received 200 mg of lyophilized powder of *E. jambolana*, and group V received 400 mg of aqueous extract of *T. cordifolia* and group VI received 200 mg/day of alcoholic extract of *M. pruriens*. The doses of the plant extracts were decided on the basis of a previous study. All the extracts were given PO every day till the end of experiment (up to day 50). Animals described as fasting had been deprived of food for at least 16 hr but given water *ad libitum*.

**Plasma glucose**—Glucose levels were estimated by commercially available glucose kits based on glucose oxidase method on every 10 day for 50 days (Autopak®, Bayer Diagnostics, Baroda).

**Tail flick latency (TFL)**—On day 50 of the experiment, TFL was studied as a parameter to assess diabetic neuropathy on electrical analgesiometer (Techno, India), before and after morphine injection (5mg/kg; sc) at 30 and 60 min intervals. Maximum latency period for the mice to flick its tail was fixed at 15 sec lest the mouse burnt its tail. Index of analgesia (%) in mice was calculated by the following formula:

\[
\text{Index of analgesia} (\%) = \frac{\text{Test Latency} \times 100}{\text{Basal Latency}}
\]

**Rate of transit of charcoal in GIT**—It was determined on day 50 of the experiment by forced feeding of charcoal (12.5%) and gum acacia (12.5%) 18. Ten min after feeding charcoal, animals were sacrificed and intestine was removed. Total length of intestine and distance travelled by charcoal in the intestine were measured and GTT was calculated as distance travelled by charcoal in the intestine divided by total length of intestine.

**Statistical analysis**—The results were analyzed for statistical significance by Student’s t test using computerised software, GraphPAD, InStat, 1990, Version 1.14, INSERM 920666S, India.

**Results and Discussion**

Aim of the present work was to study the effect of 50 days of persistent hyperglycemia on TFL (neuropathy) and rate of transit of charcoal in GIT (gastropathy) and whether 4 plant extracts (*M. charantia*, *E. jambolana*, *M. pruriens* and *T. cordifolia*) modify the outcome favorably. In addition, modification of analgesic effect of morphine and glucose levels were also assessed in the same. Effect of administration of plant extracts of *M. charantia*, *E. jambolana*, *M. pruriens* and *T. cordifolia* for 50 days in STZ diabetic mice is given in Table 1. Total reduction of blood glucose was of 24.4, 20.84, 9.07 and 7.45 % respectively.

Effect of administration of plant extracts on rate of transit of charcoal in GIT in STZ diabetic mice has been shown in Table 2. There was a significant difference in mean rate of transit in diabetic control vs normal control (83.00 ± 6.38 vs 99.16 ± 1.32%
Non-diabetic control; T. cordifolia. Group Day DC 90.3 ± 2.1 437.05 ± 12.8** 441.1 ± 19.9** 471.3 ± 19.3** 470.5 ± 12.3** 450.5 ± 10.03** 439.5 ± 14.2**

Diabetic control was compared with normal, and experimental groups were compared with their own corresponding values on day 10. NC — Non-diabetic control; DC — diabetic control; MC — M. charantia; EJ — E. jambolana; MP — M. pruriens; TC — T. cordifolia. Significant at *P < 0.05, **< 0.0001, ***< 0.00001 respectively). This finding is consistent with the previous finding\(^9\) that gastric emptying rate is faster in hypoglycemic state and slower in case of hyperglycemic state. Hyperglycemia\(^9\) has been implicated as the main factor responsible for delay in gastric emptying though direct effects of metabolic derangement via an altered autonomic function (i.e., neuropathy) cannot be ruled out\(^1\). M. charantia and E. jambolana caused a significant increase in gastric transit percentage with comparison to diabetic controls (89.28 ± 5.41 and 90.20 ± 5.20 versus 83.00 ± 6.38 respectively). M. pruriens and T. cordifolia had no such effect. Although M. charantia and E. jambolana caused a significant decrease in serum sugar levels (24 and 21% reduction respectively), an euglycemic state was not achieved.

### Table 2 — Effect of STZ and plant extracts on gastrointestinal transit time in mice

<table>
<thead>
<tr>
<th>Groups</th>
<th>Rate of transit of charcoal in GIT (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal</td>
<td>99.16 ± 13.32</td>
</tr>
<tr>
<td>Diabetic Control</td>
<td>83.00 ± 6.38**</td>
</tr>
<tr>
<td>M. charantia</td>
<td>90.28 ± 5.41*</td>
</tr>
<tr>
<td>E. jambolana</td>
<td>90.20 ± 5.20*</td>
</tr>
<tr>
<td>M. pruriens</td>
<td>80.60 ± 5.24</td>
</tr>
<tr>
<td>T. cordifolia</td>
<td>83.60 ± 5.20</td>
</tr>
</tbody>
</table>

Diabetic control was compared with the normal, and experimental groups were compared with diabetic control. Significant at *P < 0.05 and **< 0.005.

### Table 3 — Effects of plant extracts on TFL in mice (in sec)

<table>
<thead>
<tr>
<th>Groups</th>
<th>0 min</th>
<th>30 min</th>
<th>60 min</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>6.11 ± 1.09</td>
<td>9.01 ± 1.8</td>
<td>12.05 ± 0.7</td>
</tr>
<tr>
<td>Diabetic</td>
<td>10.61 ± 0.9*</td>
<td>11.8 ± 1.6</td>
<td>12.7 ± 1.4</td>
</tr>
<tr>
<td>M. charantia</td>
<td>7.95 ± 0.9</td>
<td>10.52 ± 1.2</td>
<td>13.41 ± 1.5</td>
</tr>
<tr>
<td>E. jambolana</td>
<td>8.5 ± 1.3</td>
<td>9.85 ± 1.7</td>
<td>12.55 ± 1.3</td>
</tr>
<tr>
<td>M. pruriens</td>
<td>8.92 ± 0.5</td>
<td>9.71 ± 1.2</td>
<td>12.0 ± 0.9</td>
</tr>
<tr>
<td>T. cordifolia</td>
<td>8.5 ± 1.3</td>
<td>11.55 ± 1.7</td>
<td>13.56 ± 1.3</td>
</tr>
</tbody>
</table>

Diabetic control was compared with normal and experimental groups were compared with diabetic control. Significant at *P < 0.05.

Thus, it is likely that M. charantia and E. jambolana normalised rate of transit of charcoal in GIT through an unknown independent mechanism other than its anti-hyperglycemic effect.

Effect of administration of plant extracts on TFL of STZ diabetic mice is shown in Table 3 and index of analgesia has been shown in Table 4. TFL was significantly higher in diabetic mice (10.61 ± 2.98) as compared to normal control (6.11 ± 1.09) before administration of morphine. These results are in agreement with the previous findings\(^21\). However, decrease in hot plate latency\(^24\) as well as no change has also been described earlier\(^25,26\). In the present study, TFL in treated groups before the administration of morphine was insignificantly lesser than diabetic controls. Daily administration of M. charantia and E. jambolana insignificantly prevented the rise in basal
TFL in comparison to diabetic controls (Table 3). Insulin has been shown to reverse the blunting of antinociceptive effect of morphine seen in diabetic animals\(^\text{10}\) possibly by decreasing opioid receptor affinity or change in the configuration caused by hyperglycemia\(^\text{15}\). Though the plant extracts (particularly \textit{M. charantia} and \textit{E. jambolana}) caused a significant reduction in glucose levels, it was not enough to normalize the rise seen in basal TFL in treated groups.

References