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ISOLATION OF A PROMISING ANTIDIABETIC COMPOUND FROM THE LEAVES OF TETRACERA INDICA MERR., AND IN VIVO TOXICOLOGICAL STUDIES IN DIABETES INDUCED EXPERIMENTAL ANIMALS

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Diabetes mellitus is a chronic disorder together with other metabolic abnormalities, usually developed due to insulin resistance or deficiency as well as increased hepatic glucose output [1]. The prevalence of diabetes mellitus among Malaysians adults more than 30 years of age has escalated by more than two fold over a 20-year period [2].

On the other hand, many plants have been used for the treatment of diabetes mellitus in modern system of medicine and in other ancient systems of the world. As a result, plants are now-a-days regarded as promising and significantly attractive natural sources to enrich the current therapy options against diabetes. However, so far, not many active agents have been successfully isolated and extensively studied [3]. Hence, we prompted to evaluate the antidiabetic potential of a flavonoid (5,7-dihydroxy-8-methoxyflavone [4], Figure 1) isolated from the leaves of Tetracera indica Merr., a Malaysian medicinal plant which is traditionally used to treat diabetes in Malaysia.

The study was carried out using Sprague-Dawley rats (diabetic as well as normal) to evaluate antidiabetic potential of 5,7-dihydroxy-8-methoxyflavone at three different concentrations, viz. 1 mg/kg b.w., 5 mg/kg b.w. and 25 mg/kg b.w. At 5 mg/kg b.w. and 25 mg/kg b.w., it was found to exhibit significant anti-hyperglycemic activity in alloxan induced diabetic rats and in normal rats, no hypoglycemic activity was observed at all concentrations, when compared with +ve and –ve controlled groups. The antidiabetic activity was found to be comparable with glibenclamide (GLBC), a known oral hypoglycemic agent (50 mg/kg b.w.) (Table 1). The LD₅₀ of 5,7-dihydroxy-8-methoxyflavone was found to be more than 500 mg/kg b.w. and no lethal toxicity was observed within this range. Three weeks later, the in-vivo study, histopathology of kidney and pancreas from alloxan-induced rats demonstrated the clinical manifestation of diabetic affected kidney and pancreas (Figures 2 & 3). It is concluded that the 5,7-dihydroxy-8-methoxyflavone from the leaves of T. indica is a safe and promising antidiabetic agent that could prove useful in the management of diabetes and might also provide lead for the synthesis of a new class of safe antidiabetic drugs.

Keywords: 5,7-dihydroxy-8-methoxyflavone, Tetracera indica, antidiabetic agent
Fig. 1: Structure of isolated flavonoid (5,7-dihydroxy-8-methoxyflavone) from the leaves of *Tetracera indica* Merr.

Table 1. Effect of different doses of 5,7-dihydroxy-8-methoxyflavone on blood glucose levels (mmol/L) in normal and diabetic rats at different intervals (days). The values represent the standard error of mean (S.E.M.). *indicates a significant change in blood glucose level (p<0.005).

<table>
<thead>
<tr>
<th>GROUPS</th>
<th>DAY 1</th>
<th>DAY 3</th>
<th>DAY 5</th>
<th>DAY 7</th>
<th>DAY 9</th>
<th>DAY 11</th>
<th>DAY 13</th>
<th>DAY 15</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal Healthy</td>
<td>6.2±0.6</td>
<td>6.7±0.7</td>
<td>6.1±0.3</td>
<td>5.8±0.4</td>
<td>6.1±0.5</td>
<td>6.0±0.2</td>
<td>6.5±0.4</td>
<td>5.7±0.6</td>
</tr>
<tr>
<td>Normal/Mflavone 5mg/kg b.w.</td>
<td>5.4±0.5</td>
<td>6.1±0.9</td>
<td>5.7±0.3</td>
<td>6.5±0.9</td>
<td>6.4±0.7</td>
<td>5.6±0.4</td>
<td>5.9±0.4</td>
<td>5.9±0.4</td>
</tr>
<tr>
<td>Diabetic/Mflavone 5mg/kg b.w.</td>
<td>32.8±2.4 *</td>
<td>30.4±2.2 *</td>
<td>27.5±2.0 *</td>
<td>26.9±2.2 *</td>
<td>24.7±2.5 *</td>
<td>22.6±1.8 *</td>
<td>20.1±2.8 *</td>
<td>18.6±2.7 *</td>
</tr>
<tr>
<td>Diabetic/GLBC 50mg/kg b.w.</td>
<td>30.9±1.8 *</td>
<td>29.2±1.5 *</td>
<td>25.7±1.3 *</td>
<td>22.7±1.2 *</td>
<td>20.0±1.7 *</td>
<td>17.0±2.0 *</td>
<td>13.9±1.9 *</td>
<td>10.6±1.7 *</td>
</tr>
<tr>
<td>Normal/Mflavone 25mg/kg b.w.</td>
<td>6.7±0.3</td>
<td>6.4±0.2</td>
<td>6.0±0.4</td>
<td>5.9±0.4</td>
<td>6.2±0.6</td>
<td>5.8±0.6</td>
<td>5.3±0.4</td>
<td>5.1±0.2</td>
</tr>
<tr>
<td>Diabetic/Mflavone 25mg/kg b.w.</td>
<td>30.5±3.7 *</td>
<td>25.9±7.8 *</td>
<td>22.4±7.8 *</td>
<td>19.1±7.4 *</td>
<td>16.7±7.6</td>
<td>14.6±6.9 *</td>
<td>12.9±6.4 *</td>
<td>9.9±5.8 *</td>
</tr>
<tr>
<td>Diabetic control</td>
<td>30.9±2.5</td>
<td>29.2±4.7</td>
<td>29.4±3.5</td>
<td>27.5±3.1</td>
<td>26.1±2.2</td>
<td>25.4±1.7</td>
<td>24.7±1.6</td>
<td>25.6±1.7</td>
</tr>
<tr>
<td>Diabetic/Mflavone 1mg/kg b.w.</td>
<td>32.5±3.1 *</td>
<td>30.9±5.5 *</td>
<td>30.3±5.3 *</td>
<td>29.0±4.9 *</td>
<td>28.4±4.9 *</td>
<td>27.7±4.9 *</td>
<td>26.9±4.7 *</td>
<td>26.1±4.7 *</td>
</tr>
</tbody>
</table>

**Fig. 1:** Diabetic group of rats treated with high dose of Mflavone (25 mg/kg b.w.) showing mild segmental mesangial matrix expansion from the glomeruli with partial hyalinization of glomerulus (kidney section)

**Fig. 2:** Diabetic group of rats treated with high dose of Mflavone (25 mg/kg). After 3 weeks of study, the pancreas tissue- the lighter staining tissue within the lobules represent the endocrine component of the pancreas, the pancreatic Islets of Langerhans and restoration of normal cellular population size of islets of Langerhans and less damage to islets
Currently, phytochemical databases that store information on ethnobotanical plants, compounds and its uses in Asia are limited and do not comprehensively cover the extent of digitally available information for specific species. Asia and its rich ethnobotanical medicine and associated heritage encompass a multitude of cultures, ranging from Chinese and Ayurvedic to Indo-Malay traditional medicine. Although there are an abundance of data and information, they are scattered with only a few specific databases that cater for ethnobiology and vast information available in textual format due to its historical nature. Therefore, an establishment of a comprehensive ethnobotanical database will facilitate information gathering and preserve the biocultural knowledge that is facing extinction due to habitat loss and modern development. This database Phyknome will enable researchers and public to seek and identify ethnobotanical information based on a species scientific name, descriptions and phytochemical information. Up until now, Phyknome hosts more than 22,000 plant species with 14 Divisions, 18 Class, 745 Families and more than 28,000 Genus. Plant scientific names were cross-checked with International Plant Index (IPNI) for verification. It is constructed using a digitization pipeline that allow high throughput digitization of archival data, an automated data miner to mine for pharmaceutical compounds information and an online database to integrated these information. Active compounds structure and publication information is mined from PubChem and PubMed database respectively. The pipeline is integrated with android smartphone application that can be used to report occurrences and obtain ethnobotanical data on-the-go. The main functions include an automated taxonomy, search query based on chemical compound, geolocation and bibliography. We believe that Phyknome will contribute to the digital knowledge ecosystem to elevate access and provide tools for ethnobotanical research and contributes to the management, assessment and stewardship of biodiversity. The database is available at http://mapping.fbb.utm.my/phyknome/.

Keywords: Ethnobotany, phytochemicals, databases
Fig. 1: Phyknome homepage; contain featured researchers, featured plant species, plant species counter, login page

Fig. 2: Example species page; shows accepted names (based on International Plant Index (IPNI)), synonym(s), vernacular name(s), Chinese name, taxonomic classifications

Fig. 3: Example data information; plant usage, parts used, precaution, other uses, active compound, chemical structure from PubChem, retrieved PubMed publications, other references resource

Fig. 4: Geographical information; based on GPS coordinate and retrieved from GBIF API species distribution