Preventive effects of monomethyl fumarate and a methanolic *Fumaria indica* extract against chronic foot shock stress triggered ulcers and other pathologies in rats

Presented by: Anshul Shakya

XIXth International Congress "Phytopharm 2015"
New Phytotherapeutics – Developments, Requirements and Success for Patients with Rational Phytotherapy and Courses "GxP in biomedical research"
Organized by Bonn, Germany on July 21-24, 2015
Preventive effects of monomethyl fumarate and a methanolic *Fumaria indica* extract against chronic foot shock stress triggered ulcers and other pathologies in rats

Anshul Shakya¹,*; Upendra Kumar Soni²; Geeta Rai²; Shyam Sunder Chatterjee³; Vikas Kumar¹

¹Neuropharmacology Research Laboratory, Department of Pharmaceutics, Indian Institute of Technology (Banaras Hindu University), Varanasi-221 005, India
²Department of Molecular and Human Genetics, Faculty of Science, Banaras Hindu University, Varanasi, India
³Stettiner Str.1, D-76138 Karlsruhe, Germany
*Current Address: Department of Pharmaceutical Sciences, Dibrugarh University, Dibrugarh-786 004, Assam, India
Introduction

*Fumaria indica* Linn., (Fumariaceae)

– a wildly growing weed
– traditionally medicinal plant widely used for diverse therapeutic purposes in Ayurveda and other traditionally known medical systems of India and other Asiatic countries.

Standardized extracts of *Fumaria indica* for fumaric acid and its methyl ester has adaptogenic like potential including:

– anti-stress
– anti-amnestic
– Anxiolytic
– Antidepressant
– anti-ulcer
– analgesic and anti-inflammatory efficacy
Fumaric acid and its methyl esters viz. mono-methyl and di-methyl fumarate has been well known for protective effect against inflammatory disorders such as psoriasis, multiple sclerosis and Huntington's disease due to its inhibitory effect over inflammatory cytokines release.
Objective

• To reaffirm that fumarates are quantitatively the major chronic stress response suppressing bioactive constituents of medicinally used *Fumaria indica* extracts.
Materials and Methods

• Male adult Charles Foster albino rats (150 ± 10 g) were obtained from Central Animal House of the Institute of Medical Sciences, Banaras Hindu University, Varanasi, India (Registration Number: 542/AB/CPCSEA).

• Prior approval from the Central Animal Ethical Committee (CAECU) of Banaras Hindu University, Varanasi, India, was obtained for the study protocols (Dean/11-12/CAEC/324, dated 30-11-2011).
• Standardized 50% methanolic extract of *Fumaria indica* (MFI) was generously supplied as well as analytically characterized by the Indian Herbs Research and Supply Co. Ltd. Saharanpur, India.

• Analytically estimated content of free fumaric acid in this extract was 0.59% (w/w) and that of the conjugates of the acid was 0.48% (w/w).

• Pharmaceutical quality of *Withania somnifera* (WS) root extract, analytically characterized to contain withanolide aglycones and glycosides (2.6 %, w/w), and withaferin A (0.7 %, w/w) by HPLC.

• Mono-methyl fumarate (MMF) and was procured from Sigma-Aldrich, USA.
• Nine groups of randomly assigned experimental groups were:
  i. Normal control
  ii. Stress control
  iii. Stress + MFI 60 mg/kg/day
  iv. Stress + MFI 120 mg/kg/day
  v. Stress + MFI 240 mg/kg/day
  vi. Stress + MMF 1.25 mg/kg/day
  vii. Stress + MMF 2.5 mg/kg/day
  viii. Stress + MMF 5 mg/kg/day
  ix. Stress + WS 100 mg/kg/day

• All oral treatments to stressed animals were given 60 min before daily 1-h stress exposures.
Chronic foot-shock stress induced ulcer

• Each individual rat of the stressed groups was subjected to 1-h daily foot-shocks through a grid floor in a standard conditioning chamber with the escape route closed.

• The duration of each unpredictable foot-shock (2 mA) and the intervals between the shocks were randomly programmed between 3 to 5 s and 10 to 110 s, respectively.

• One hour after the last stress session on day 21, blood samples were collected from retro-orbital plexus for plasma corticosterone assay and cytokine expressions.

• Thereafter, the animals were sacrificed and their brains, stomach, adrenal gland and spleen were removed to determine various parameters compiled in result section.
Statistical analysis

Data of all experiments will be expressed as Mean ± Standard Error of Mean (SEM) of six animals in each group.

Differences among different treatment groups will be determined by one way analysis of variance (ANOVA), followed by Student-Newman-Keuls test, or unless stated otherwise.

GraphPad Prism 5 will be used for statistical analysis.
Results and Discussion
**Body weight**

![Graph showing body weight changes](image)

**Fig. 1** Effect of methanolic extract of *Fumaria indica* (MFI), monomethyl fumarate (MMF) and *Withania somnifera* root extract (WS) on a body weight change observe between day 1 and 21. Values are Mean ± SEM (n = 6), * p<0.05 versus normal control; ¥ p<0.05 versus stressed control.
Fig. 2 Effect of MFI, MMF and WS on a weight of adrenal glands, b weight of spleen, and c plasma corticosterone level of chronic foot shock stressed rats. Values are Mean ± SEM (n = 6), * p<0.05 versus normal control; ¥ p<0.05 versus stressed control.
Fig. 3 Pictorial and histological sections (Haematoxylin and Eosin staining, 10X) of isolated stomachs depicting (red arrow for ulcer and blue arrow for mucosal erosion) the effect of methanolic extract of *Fumaria indica* (MFI), monomethyl fumarate (MMF) and *Withania somnifera* root extract (WS) treatments on chronic foot shock stress induced ulcers in rats. a vehicle-treated control, b vehicle treated stress control, c MFI (60 mg/kg), d MFI (240 mg/kg), e MFI (240 mg/kg), f MMF (1.25 mg/kg), g MMF (2.50 mg/kg), h MMF (5.00 mg/kg), and i WS (100 mg/kg)
Table 1. Effect of methanolic extract of *Fumaria indica* (MFI), monomethyl fumarate (MMF) and *Withania somnifera* root extract (WS) on chronic foot shock stress induced gastric ulceration in rats

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Number of ulcer (N)</th>
<th>Severity of ulcers (Score)</th>
<th>Ulcer index (%)</th>
<th>% Protection</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal control (vehicle)</td>
<td>0.00</td>
<td>0.00</td>
<td>0.00</td>
<td>-</td>
</tr>
<tr>
<td>Stress control (vehicle)</td>
<td>7.17±0.40*</td>
<td>19.50±0.76*</td>
<td>26.67±1.15*</td>
<td>0.00</td>
</tr>
<tr>
<td>Stress+ MFI 60 mg/kg/day</td>
<td>5.50±0.43¥</td>
<td>12.50±0.96¥</td>
<td>18.00±1.17¥</td>
<td>32.51</td>
</tr>
<tr>
<td>Stress+ MFI 120 mg/kg/day</td>
<td>3.67±0.33¥</td>
<td>8.00±0.78¥</td>
<td>11.67±1.02¥</td>
<td>56.26</td>
</tr>
<tr>
<td>Stress+ MFI 240 mg/kg/day</td>
<td>2.17±0.31¥</td>
<td>5.17±0.79¥</td>
<td>7.33±1.09¥</td>
<td>72.50</td>
</tr>
<tr>
<td>Stress+ MMF 1.25 mg/kg/day</td>
<td>6.00±0.37¥</td>
<td>13.33±0.62¥</td>
<td>19.33±0.92¥</td>
<td>27.51</td>
</tr>
<tr>
<td>Stress+ MMF 2.5 mg/kg/day</td>
<td>4.17±0.48¥</td>
<td>10.33±0.84¥</td>
<td>14.50±1.31¥</td>
<td>45.63</td>
</tr>
<tr>
<td>Stress+ MMF 5.0 mg/kg/day</td>
<td>2.83±0.31¥</td>
<td>7.17±0.48¥</td>
<td>10.00±0.78¥</td>
<td>62.50</td>
</tr>
<tr>
<td>Stress+ WS 100 mg/kg/day</td>
<td>1.83±0.17¥</td>
<td>4.50±0.56¥</td>
<td>6.33±0.72¥</td>
<td>76.25</td>
</tr>
</tbody>
</table>
Fig. 4 Effect of MFI, MMF and WS on a LPO level, b SOD activity, c Catalase activity, and d GSH level in fundus of stomach. Values are Mean ± SEM (n = 6), * p<0.05 versus normal control; ¥ p<0.05 versus stressed control.
Table 2 Effect of MFI, MMF and WS on weight of glandular portion of stomach and mucosal parameters in chronic foot shock stress rats.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Weight of glandular stomach (mg/100 g, BW)</th>
<th>Mucosal parameters</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>TP (µg/100 mg tissue)</td>
</tr>
<tr>
<td>Normal control (vehicle)</td>
<td>560.4±11.1</td>
<td>392.20±4.41</td>
</tr>
<tr>
<td>Stress control (vehicle)</td>
<td>416.0±8.5*</td>
<td>386.23±5.49</td>
</tr>
<tr>
<td>Stress+ MFI 60 mg/kg/day</td>
<td>455.1±12.0¥</td>
<td>380.30±6.24</td>
</tr>
<tr>
<td>Stress+ MFI 120 mg/kg/day</td>
<td>514.8±15.0¥</td>
<td>382.64±3.69</td>
</tr>
<tr>
<td>Stress+ MFI 240 mg/kg/day</td>
<td>546.6±10.6¥</td>
<td>379.63±6.01</td>
</tr>
<tr>
<td>Stress+ MMF 1.25 mg/kg/day</td>
<td>438.9±10.7</td>
<td>376.28±4.85</td>
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<tr>
<td>Stress+ MMF 2.5 mg/kg/day</td>
<td>509.8±7.7¥</td>
<td>381.43±11.90</td>
</tr>
<tr>
<td>Stress+ MMF 5.0 mg/kg/day</td>
<td>535.4±11.14¥</td>
<td>378.49±7.17</td>
</tr>
<tr>
<td>Stress+ WS 100 mg/kg/day</td>
<td>557.0±13.4¥</td>
<td>371.07±8.85</td>
</tr>
</tbody>
</table>
Fig. 5 Effect of MFI, MMF and WS on monoamine (5-HT, NE and DA) levels in brain (HIP, HYP and FC). a 5-HT level in HIP, b 5-HT level in HYP, c 5-HT level in FC, d NE level in HIP, e NE level in HYP, f NE level in FC, g DA level in HIP, h DA level in HYP, i DA level in FC.
Fig. 6 Effect of MFI, MMF and WS on a LPO level, b SOD activity, c catalase activity, and d GSH level in frontal cortex (FC) of brain.
Fig. 7 Effect of MFI, MMF and WS on expressions of TNF-α, IL-10 and IL-1β in chronic stressed rats. Gel picture showing expression of cytokines normalized with β-actin housekeeping gene in a blood and c brain, and percentage change in mRNA expression of cytokines in b blood (WBC), and in d frontal cortex.
Conclusion

Fumarates are quantitatively the major bioactive constituents of *Fumaria indica* extracts involved in their preventive effects against chronic stress triggered pathologies.

Reported observations strongly suggest that daily intake of relative low oral doses (<1.25 mg/kg/day) of pure monomethyl fumarate could be an effective therapeutic alternative for prevention of chronic stress triggered gastric ulcers and other pathologies.
Bibliography


Acknowledgement

• Indian Herbs Research and Supply Co. Ltd. Saharanpur, India

• Natural Remedies Pvt. Ltd., Bangalore, India

• Dibrugarh University, Dibrugarh, Assam

• Organiser “PHYTOPHARM – 2015”
Thanking you!