PHYTOCHEMICAL AND NEUROPROTECTIVE INVESTIGATION ON FERULA HERMONIS

Karim Raafat
Department of Pharmaceutical Sciences, Faculty of Pharmacy, Beirut Arab University, Lebanon,
k.raafat@bau.edu.lb

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Abstract
Ferula hermonis (F. hermonis, Fh) is one of the important medicinal-plants cultivated in Lebanon. The aim of this study is to phytochemically-investigate F. hermonis and its most active compound(s) and to explore their neuroprotective potentials utilizing an in vivo model of glycine receptor. HPLC-DAD investigation was done to identify F. hermonis phytochemical constituents. Combined chromatographic, bio-guided fractionation, and in vivo model of glycine receptor were utilized to identify its most active constituent(s). HPLC has shown that F. hermonis has shown 11 major peaks identified as: (1) Jaeskenin (2.47%), (2) Acetoxy-Ferutinin (3.71%), (3) Lapiferin (4.95%), (4) Siol anisate (6.16%), (5) Fertidin (7.38%), (6) Ferutinin (24.60%), (7) p-Coumariloxy jaekeanadiol (7.29%), (8) Akiferin (5.98%), (9) Ferulenol (23.7%), (10) Ferutidin (4.68%), and (11) Jaekeanadiol benzoate (6.03%). Ferutinin (Ft) was identified as the most active constituent in F. hermonis. The novelty in this work that F. hermonis has shown neuroprotective potentials utilizing an ICV, for the first time, in the in-vivo model of glycine receptor. Ft has shown more significant (p < 0.05) neuroprotective potentials than F. hermonis. It could be concluded that F. hermonis has significant neuroprotective potentials and that Ft might be responsible for this activity.

Keywords
Ferula hermonis; Phytochemical Content; Analysis; Ferutinin; Neuroprotective.
PHYTOCHEMICAL AND NEUROPROTECTIVE INVESTIGATION ON FERULA HERMONIS

K. RAFAFAT

1 Karim Raafat, Department of Pharmaceutical Sciences, Faculty of Pharmacy, Beirut Arab University, Lebanon

ABSTRACT: Ferula hermonis (F. hermonis, Fh) is one of the important medicinal-plants cultivated in Lebanon. The aim of this study is to phytochemically-investigate F. hermonis and its most active compound(s) and to explore their neuroprotective potentials utilizing an in vivo model of glycine receptor. HPLC-DAD investigation was done to identify F. hermonis phytochemical constituents. Combined chromatographic, bio-guided fractionation, and in vivo model of glycine receptor were utilized to identify its most active constituent(s). HPLC has shown that F. hermonis has shown 11 major peaks identified as: (1) Jaeskenin (2.47%), (2) Acetoxy-Ferutinin (3.71%), (3) Lapiferin (4.95%), (4) Siol anisate (6.16%), (5) Fertidin (7.38%), (6) Ferutinin (24.60%), (7) p-Coumariloxy jaekeanadiol (7.29%), (8) Akiferin (5.98%), (9) Ferulenol (23.7%), (10) Ferutidin (4.68%), and (11) Jaekeanadiol benzoate (6.03%). Ferutinin (Ft) was identified as the most active constituent in F. hermonis.

The novelty in this work that F. hermonis has shown neuroprotective potentials utilizing an ICV, for the first time, in the in-vivo model of glycine receptor. Ft has shown more significant (p˂ 0.05) neuroprotective potentials than F. hermonis. It could be concluded that F. hermonis has significant neuroprotective potentials and that Ft might be responsible for this activity.

KEYWORDS: Ferula hermonis; Phytochemical Content; Analysis; Ferutinin; Neuroprotective.

1. INTRODUCTION

Medicinal plants traditionally have shown superiority in the management of several disorders, especially the neurological and chronic disorders (Deventer, Pozo, Van Eenoo, & Delbeke, 2007).

Ferula hermonis (F. hermonis, F. Apiaceae) is one of the important medicinal plants cultivated in Lebanon (Raafat, 2016; Raafat & El-Lakany, 2015). F. hermonis comprises several phytochemical compounds that have not been widely explored. F. hermonis has been traditionally used for erectile dysfunction and several neurological disorders (Raafat, 2013). Phytochemical analyses have shown that F. hermonis comprises daucane-aryl-esters like ferutinin (Ft) (Galal, 2000; Lhuillier et al., 2005). Ft has various pharmacological-activities including estrogenic and hypoglycemic activities (Raafat, 2013). Ft is one of the potent natural phyto-estrogen which has potentiating-activity on estrogen-receptors, especially alpha-receptor. It seems that Ft plays an important role in F. hermonis pharmacological-activities, chiefly the useful-effects of this plant on diabetes, impotence, osteoporosis and inflammation (Al-Ja'fari et al., 2011; Allouh, 2011; Geroushi et al., 2011; Ibrahim et al., 2012; Sattar & Iranshahi, 2017).

Glycine receptor is one of the important ion channels responsible for neuro-stability. It possesses an inhibitory response to neuron cells guarding against neurotoxicity (Raafat, Breitinger, Mahran, Ayoub, & Breitinger, 2010). Intracerebroventricular (ICV) procedures have been established to be a distinguished in vivo method in discovering GlyR-modulators (Raafat & Wurglics, 2018).

Therefore, the aim of this study is to phytochemically-investigate F. hermonis and its most active compound(s) and to explore their neuroprotective potentials utilizing an ICV in vivo model of glycine receptor.

2. EXPERIMENTAL

2.1 Standards and Solvents

All standards and solvents (analytical grade) have been purchased from Sigma-Aldrich (Germany) (Saleh, El-Darra, Raafat, & El Ghazzawi, 2018).
2.2 Extraction
Ferula hermonis roots were acquired from Ibn Al-Nafess (Lebanon). The roots have been compared to an original sample, and a representative aliquot has been placed in the Faculty herbarium with a voucher-specimen no. (PS-17-35).

The roots were sonicated for 3h with acetone and then filtered, and dried using a rotary-evaporator (40°C under-vacuum), and the extract was kept under -4 °C until further use.

2.3 Chromatography
2.3.1 RP-HPLC method
The Jasco HPLC system (Japan) (Raafat, Wurglics, & Schubert-Zsilavecz, 2016) consisted of a pump and a C18 stationary phase column was used for chromatographic separations. The eluent comprised MeOH/Phosphate buffer pH 2.41 (50:50 v/v) at flow-rate of one mL/min at 202 nm maintained at 40°C.

2.3.2 Phytochemical bio-guided fractionation and identification method
F. hermonis extract has been fractionated utilizing a preparative column-chromatography (CC) (50 mm * 100 cm) utilizing RP-silica-gel and eluted in elevating polarity with MeOH-Ethyl acetate (0:100, 5:95, 10:90, 20:80, 30:70, 50:50,70:30, 80:20, 90:10, 100:0v/v). During the whole RP-CC process the mobile phase has been collected by time in a sequence of more than two hundred fractions. Similar samples were combined and concentrated and each fraction has been examined in a similar fashion as the whole-extract utilizing an in vivo model of glycine receptor.

2.4 Animals
After 7 days of accommodation in standard-cages, male Swiss-Webster mice (BAU, Lebanon) have had open-entrance to standard food and water. Animal-care and experiments were done abiding by the animal experiment-legislations and with the approval of BAU-Institutional Review Board (2014A-004-P-R-0006).

2.5 Loss of the Righting Reflex (LORR) in vivo Experimentation
It was described before that the extract/active compound combined with glycine (gly) when administered intracerebroventricularly (ICV) might augment the EtOH central-inhibitory activity if the extract/active compound was GlyR potentiator, and inhibited with GlyR blockers like strychnine (Raafat & Wurglics, 2018; Williams Kl Fau - Ferko, Ferko Ap Fau - Barbieri, Barbieri Ej Fau - DiGregorio, & DiGregorio, 1995).

In the EtOH presence, gly alone or combined with Fh (25, 50 or 100 mg/kg), or Ft (6.25, 12.5 or 25 mg/kg) have been ICV-injected. The approach of this procedure was that Fh or Ft might potentiate or block gly-potentiated EtOH central-depressant activities. Strychnine, a GlyR blocker, was ICV-injected after the administration of gly alone or combined to verify Fh or Ft neuroactive mechanism.

Bicuculline (Bic), a GABAA-receptor inhibitor, was ICV-infused after the administration of gly alone or combined to assure that GlyR is the only operating receptor (Williams Kl Fau - Ferko et al., 1995).

The loss of righting reflex (LORR) has been utilized to evaluate the level of central-inhibition produced by EtOH using a procedure reported before (Raafat & Wurglics, 2018; Williams Kl Fau - Ferko et al., 1995). In brief, each animal (n=4/group) received EtOH (20%, 4.0 gm/kg) as an intraperitoneal (i.p.) injection intended to LORR-induction (Wallgren & Barry, 1970). The time to LORR (seconds), is the duration from injection to the EtOH-induced LORR. Whilst the LORR-end (min.), is the capability of the animals to right themselves by rotating-backward to their paws 3 times in 15 seconds when located backwards. Furthermore, the EtOH-LORR is the time between the initial-LORR and the subsequent righting-reflex (RR) regaining was reported. After test ICV-injections, a second-period of LORR was recorded. This 2nd-period was recognized as the return to LORR, which is the period between ICV-injection and the subsequent regaining of RR.

2.6 ICV Extract/Active Constituent Administration
After 20 min after the animal lost the RR, a sagittal incision has been done on the posterior-part of the animal-head revealing the skull-sutures. Previously EtOH-anesthetized animal an opening three mm in depth was made two mm dorsal to the bregma-suture and two mm dorsal to the sagittal-suture utilizing a twenty four gauge-needle. Following the mice regaining the RR after the injection of EtOH, they had an intracerebroventricular-injection over a period of ten seconds of saline or extract/active constituent (with a total volume of ten µl). Consequent with ICV-injection of saline or extract/active constituent, a second-LORR has been reported (return to LORR) (Lundquist, 1959; Raafat & Wurglics, 2018).
2.7 Statistics
The data were recorded as means ± SEM. Upon correlating all values recorded to the control, all test have been determined utilizing one way ANOVA test, setting p-values < 0.05 as statistically significant (Ikeda, Dohi, & Tsujimoto, 1982; Raafat & Wurglics, 2018; Sawaki et al., 2000).

3. RESULTS AND DISCUSSION
3.1 Chromatography
3.1.2 RP-HPLC phytochemical standardization method
Utilizing the optimized RP-HPLC conditions used in this study, the F. hermonis extract has revealed 11 major peaks: (1) Jaeskenin (2.47%), (2) Acetoxy-Ferutinin (3.71%), (3) Lapiferin (4.95%), (4) Siol anisate (6.16%), (5) Fertidin (7.38%), (6) Ferutinin (24.60%), (7) p-Coumariloxy jaekeanadiol (7.29%), (8) Akiferin (5.98%), (9) Ferulenol (23.7%), (10) Ferudin (4.68%), and (11) Jeaekanadiol benzoate (6.03%). (Fig. 1). This method has shown to be an efficient method in separation and identification of F. hermonis extract.

Fig. 1 HPLC-DAD of Ferula hermonis: (1) Jaeskenin (2.47%), (2) Acetoxy-Ferutinin (3.71%), (3) Lapiferin (4.95%), (4) Siol anisate (6.16%), (5) Fertidin (7.38%), (6) Ferutinin (24.60%), (7) p-Coumariloxy jaekeanadiol (7.29%), (8) Akiferin (5.98%), (9) Ferulenol (23.7%), (10) Ferudin (4.68%), and (11) Jeaekanadiol benzoate (6.03%). The eluent was methanol: pH 2.41 phosphate-buffer (50:50) utilizing 202 nm at 40° C.

3.2 Phytochemical Bio-guided Fractionation and Identification Method
Depending on the bio-guided isolation and fractionation procedures, the most-active part of F. hermonis extract was recognized using RP-HPLC procedure and in vivo model of glycine receptor. The most active fraction major peak was recognized as ferutinin (Ft), utilizing standard Ft steeping-method (Raafat et al., 2016).
3.3 Righting Reflex Loss (LORR) Test

Animals have shown LORR subsequent to EtOH i.p. injection. The LORR onset was established at 82.98 ± 5.50 s, and EtOH LORR endured for 33.58 ± 2.30 min. Glycine (gly) has potentiated EtOH CNS-depressant activities in animals, as marked by the dose dependant and immediate return to the LORR after gly-ICV injection at different doses (Table 1).

The results in (Table 1) showed that when different doses of strychnine (STR) (50, 100, or 300 nmol/kg) were ICV-injected in combination with EC50 gly (15 μmol/kg), STR has blocked gly-activities in a dose-dependent fashion.

STR ICV-injection (300 nmol/kg) has totally blocked the gly-activities, suggesting that the strychnine-sensitive GlyR is the only functioning receptor (Table 1).

The data recorded have shown that different doses of Fh (25, 50 and 100 μmol/ kg) in combination with gly 15 μmol/ kg, Fh has potentiated gly-effects in a dose-dependent fashion, implying that Fh is a GlyR potentiator (Fig. 2).

The data have shown that different doses of Ft (6.25, 12.5 and 25 μmol/ kg) in combination with 15 μmol/ kg gly, Ft also potentiated gly-activities in a dose-dependent manner (Table 1), suggesting Ft as also a potent GlyR potentiator (Fig. 2).

STR (300 nmol/kg), the specific GlyR blocker, combined with gly and Fc (at low doses) abolished Fc potentiation responses (Fig. 2). Fh (100 μmol/kg, at the highest dose) reversed STR (300 nmol/kg) inhibition (Fig. 2), empowering the result that Fh is a GlyR potentiator, likewise, Ft (25 μmol/kg, at the highest dose) also reversed STR (300 nmol/kg) blockage (Fig. 2), supporting the results that Ft is a possible GlyR potentiator. Biologically active-materials that reverse the STR action have shown to possess anticonvulsant-activities (Hisaoka & Levy, 1985; Khisti, VanDoren, O’Buckley, & Morrow, 2003; Mascia, Machu, & Harris, 1996; Raafat & Wurglics, 2018). Thus, Fh and Ft might possess neuroprotective properties by ameliorating STR convulsing activities.

<table>
<thead>
<tr>
<th>Test Solution</th>
<th>Time to return to LORR (min.)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vehicle Control</td>
<td>0.01 ± 0.01</td>
</tr>
<tr>
<td>Gly 1 mmol/kg</td>
<td>2.70 ± 0.15</td>
</tr>
<tr>
<td>Gly 15 mmol/kg</td>
<td>13.11 ± 0.75</td>
</tr>
<tr>
<td>Gly 25 mmol/kg</td>
<td>19.00 ± 1.34</td>
</tr>
<tr>
<td>Gly 50 mmol/kg</td>
<td>27.10 ± 1.99</td>
</tr>
<tr>
<td>Gly 15 mmol/kg +STR 50 nmol/kg</td>
<td>10.70 ± 0.03</td>
</tr>
<tr>
<td>Gly 15 mmol/kg +STR 100 nmol/kg</td>
<td>2.70 ± 0.08</td>
</tr>
<tr>
<td>Gly 15 mmol/kg +STR 300 nmol/kg</td>
<td>0.40 ± 0.09</td>
</tr>
</tbody>
</table>

Thus, Fh and Ft might possess neuroprotective properties by ameliorating STR convulsing activities.
Fig. 2 Return to Righting Reflex (RR) experiment. RR following intracerebroventricular-injection of (Gly 15) Glycine 15mmol/kg, (STR 300) STR 300nmol, (Gly 15 + Fh 25) Glycine 15mmol/kg and Fh 25mmol/kg, (Gly 15 + Fh 100) Glycine 15mmol/kg and Fh 100mmol/kg, (Gly 15 + Fh 100) Glycine 15mmol/kg and Fh 100mmol/kg, (Gly 15 + Ft 6.25) Glycine 15mmol/kg and Ft 6.25mmol/kg, (Gly 15 + Ft 12.5) Glycine 15mmol/kg and Ft 12.5mmol/kg, (Gly 15 + Ft 25) Glycine 15mmol/kg and Ft 25mmol/kg, (Gly 15 + Ft 25 + STR 300) Glycine 15mmol/kg, Ft 25mmol/kg and STR 300nmol/kg, (Gly 15 + Fh 25 + STR 300) Glycine 15mmol/kg, Fh 25mmol/kg and STR 300nmol/kg, (Gly 15 + Fh 50 + STR 300) Glycine 15mmol/kg, Fh 50mmol/kg and STR 300nmol/kg, (Gly 15 + Fh 100 + STR 300) Glycine 15mmol/kg, Fh 100mmol/kg and STR 300nmol/kg, (Gly 15 + Fh 25 + STR 300) Glycine 15mmol/kg, Ft 25mmol/kg and STR 300nmol/kg, (Gly 15 + Fh 100 + STR 300) Glycine 15mmol/kg, Fh 100mmol/kg and STR 300nmol/kg, (Gly 15 + Fh 100 + STR 300) Glycine 15mmol/kg, Ft 25mmol/kg and STR 300nmol/kg, or (Gly 15 + Ft 25 + STR 300) Glycine 15mmol/kg, Ft 25mmol/kg and STR 300nmol/kg. Results are shown as means ± SEM, “*” represent a significant difference (p < 0.05).

Therefore, it could be determined that Fh and Ft were in vivo modulators to the GlyR. Accordingly, the intracerebroventricular method has proven to be a trustworthy method for the discovery of GlyR-modulators.

4. CONCLUSIONS
HPLC has shown that F. hermonis has shown 11 major peaks identified as: (1) Jaeskenin (2.47%), (2) Acetoxy-Ferutinin (3.71%), (3) Lapiferin (4.95%), (4) Siol anisate (6.16%), (5) Fertidin (7.38%), (6) Ferutinin (24.60%), (7) p-Coumariloxojaekeanadiol (7.29%), (8) Akiferin (5.98%), (9) Ferulenol (23.7%), (10) Ferutidin (4.68%), and (11) Jaekeanadiol benzoate (6.03%). Ferutinin (Ft) was identified as the most active constituent in F. hermonis. The novelty in this work that F. hermonis has shown neuroprotective potentials utilizing an ICV, for the first time, in the in-vivo model of glycine receptor. Ft has shown more significant (p < 0.05) neuroprotective potentials than F. hermonis. It could be concluded that F. hermonis has significant neuroprotective potentials and that Ft might be responsible for this activity.

CONFLICTS OF INTEREST
The author declares no conflicts of interest.
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