Exploration of the Protective Effects of Some Natural Compounds against Neurodegeneration Exploiting Glycine Receptors *in vivo* Model

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Abstract

The commencement of the presented project was by screening of natural compounds extracted from neuroactive endogenous medicinal plants. Explicitly, ferutinin (*Ferula hermonis L.*), thymoquinone (*Nigella sativa L.*), eugenol (*Syzygium aromaticum L.*) and 6-gingerol (*Zingiber officinale L.*) were tested for their protective effect against neurodegeneration utilizing Glycine receptors (GlyRs) *in vivo* model. None of these compounds were reported before to modulate the *in vivo* GlyRs. GlyRs are inhibitory key mediators of synaptic signaling in spinal cord, brain stem, and higher central nervous system regions. Neurodegeneration may cause alteration of the GlyRs causing strychnine-like convulsions and stiffness. Modulation of GlyRs *in vivo* was studied in a mouse model of strychnine toxicity. Ferutinin revealed to be potent modulators to GlyR; with potential anticonvulsant properties in low doses. Thymoquinone, eugenol and 6-gingerol when given together with strychnine, in low concentrations reduce strychnine toxicity by reversing strychnine toxicity in mice. It could be concluded that all compounds under investigation could be used as sedatives in low doses. In order to fight against neurodegenerative diseases is to improve body antioxidant, the compounds under investigation provided to be good sources for antioxidant potential. In brief, ferutinin, thymoquinone, eugenol and 6-gingerol suggested being novel GlyR modulators, good phytochemicals, pharmacological tool and a dose sensitive drug to treat stiffness, convulsions and prophylactic agents to guard against neurodegenerative disorders.

Keywords: Phytochemistry; Glycine receptors; Ferutinin; Thymoquinone; Eugenol and 6-gingerol; Antioxidant; Neurodegenerative disorders

Introduction

The use of endogenous natural products with therapeutic properties is as ancient as human civilization and, for a long time, plant products were the main sources of drugs [1]. In an attempt to find potential neuroprotective agents from plants against neurodegeneration, we examined whether a number of natural products of endogenous herbal medicines may exhibit protective efficacy on the *in vivo* GlyRs.

The selection of the natural compounds under investigation was based on screening of many neuroactive compounds, utilizing preliminary electrophysiological studies using patch-clamp. Therefore, the choice of quinone and phenolic compounds under investigation was based on their preliminary modulatory effects in the *in vitro* homomeric α1 GlyR transfected on HEK 293 cells.

There is increasing evidence for many potential benefits through quinone and phenolic compounds mediated regulation of cellular processes such as inflammation and neuroactivity [2]. Inductive or signaling properties of quinones and phenolics may occur at concentrations much lower than required for effective radical scavenging [2]. Moreover, quinones and phenolics demonstrate numerous biological and pharmacological effects, including anti-inflammatory, and anticarcinogenic [3-5].

Sesquiterpene compounds exhibit numerous biological and pharmacological effects, including antifungal and phytoestrogen [6], as well as apoptotic effect [7]. Several species of Ferula genus has been used in folk medicine in digestive disorders, rheumatism, headache, arthritis and as, antispasmodic and aphrodisiac [8]. Ferutinin (Jaeschkeanadiol p-hydroxy benzoate) (Figure 1A) is mainly found in *Ferula hermonis L.* "Zallouh", and other Ferula species (like, *Ferula tenuisecta*) [9]. It belongs to a broad group of sesquiterpene ducane esters [10] and has a wide range of biological activities. These include neuro-pharmacological actions such as tranquilizers and mild sedation. Ferutinin was found to stimulate nitric oxide synthase activity in median eminence of the rat [8].

Nigella sativa L. seeds contain 0.4%-2.5% essential oil. The major component was the quinone, thymoquinone (27.8%–57.0%) (Figure 1B) [11]. Most properties of whole seeds or their extracts are mainly attributed to quinone constituents [12]. More recently, a great deal of attention has been given to this pharmacologically active quinone. It has been shown that thymoquinone has several properties including analgesic and anti-inflammatory actions [13,14], protection against chemicals induced carcinogenesis [15], the inhibition of membrane lipid peroxidation [14], anticonvulsant activity in the petit mal epilepsy probably through an opioid receptor-mediated increase in GABAergic tone [11].

Eugenol (4-allyl-2-methoxyphenol) (Figure 1C), a phenylpropanoid compound, is a major constituent of *Syzygium aromaticum* [16]. Eugenol has anticonvulsive and hypothermic activities. It also has antioxidant properties and protects neurons in culture from toxic events. Eugenol is also widely used as an analgesic in dentistry [17]. 6-Gingerol is a pungent principle in the rhizome of ginger and possesses the labile β-hydroxy keto functional group, which makes it susceptible to transformation to less-pungent compounds such as shogaols and zingerone by elevated temperature. 6-Gingerol has been reported to exhibit many interesting pharmacological and physiological functions for example; antipyretic, cardiotonic, chemopreventive, anti-inflammatory and antioxidant properties [18,19]. However, the neuroprotective potential of 6-gingerol is still under investigation and needs to be clarified. None of these compounds were tested on the *in vivo* GlyRs.

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In our previous work, it was proved that phenolics *in vivo* and *in vitro* have potent modulatory actions on GlyRs [20,21]. The inhibitory GlyRs is a member of the cysteine loop superfamily of ligand-gated ion channel receptors. It shares structural similarity with nicotinic acetylcholine [22]. The GlyRs is chiefly expressed in spinal cord, brain stem, and other regions of the central nervous system, where it mediates rapid synaptic inhibition [22-26]. Strychnine possesses convulsion action due to interference with postsynaptic inhibition mediated by glycine. Glycine is a key inhibitory transmitter to motor neurons and interneurons in the spinal cord, and strychnine acts as a selective, competitive antagonist to block the inhibitory effects of glycine at all glycine receptors [20]. Neurodegeneration can cause alteration of the GlyRs causing strychnine-like convulsions and stiffness [27]. Flavonoids, alcohols and local anesthetics have been shown to modulate GlyRs function [20]. In this study, the aim of combination of natural compounds under investigation with strychnine (Figure 1D and 1E), is to explore the effect of these extracts and phenolics on neurodegeneration, taking GlyRs as *in vivo* model [20,27]. Such combinations were used before in traditional medicine [20,28,29]. Nevertheless, all natural compounds under investigation were not reported to modulate the *in vivo* GlyRs. The combined effects of the quinone or phenolic compounds on strychnine lethality may be synergistic or antagonistic. Accordingly, we investigated the interaction of these substances via *in vivo* strychnine lethality test. The aim of this study was to examine the protective effect of ferutinin, thymoquinone, eugenol and 6-gingerol against neurodegeneration by the manipulation of the *in vivo* GlyRs and finding their radical scavenging potential.

Materials and Methods

Plant material

Ferula hermonis, Nigella sativa, Syzygium aromaticum and Zingiber officinale were commercially purchased (Ibn-Al-Nafess herbalist, Beirut, Lebanon). The plants were identified by direct comparison with an authentic sample. The plants were kept at the Department of Pharmaceutical Sciences, Faculty of Pharmacy, Beirut Arab University, Lebanon, with authentication numbers; Ferula hermonis (PS-1310), Nigella sativa (PS-1304), Syzygium aromaticum (PS-1308) and Zingiber officinale (PS-1311).

Sample preparation

Ferula hermonis roots and seeds, Nigella sativa seeds, Syzygium aromaticum flower-buds and Zingiber officinale rhizomes were

separately dry ground using TCM grinder (TCM, China). All fine powders were extracted using hexane, ethyl acetate and 80% ethanol respectively, and were stirred for 24 hours in their liquors. During which the flasks were covered by aluminum foil to prevent the light damage. After 24 hours, the extracts were double filtered through a porcelain funnel using 20-25 μM filters. The filtered extracts were well dried using Rotavap (Buchi, Germany) at temperature 40°C under vacuum.

Reagents and chemicals

Reference Standard ferutinin, thymoquinone, eugenol, 6-gingerol, strychnine and HPLC solvents were commercially purchased (Sigma-Aldrich, Germany). All standards were dissolved in dimethyl sulphoxide (DMSO) for the *in vivo* test, and in methanol in the radical scavenging experiment.

Fractionation and isolation of the effective compound

All extracts were fractionated using column chromatography (CC). Preparative chromatography column was used. Gradient elution was employed utilizing normal phase silica gel as stationary phase. During the entire chromatography process the eluent was collected in a series of fractions by time. Fractions were analyzed using TLC and RP-HPLC compared to commercial standards. HPLC analysis was carried out in a JASCO instrument (JASCO, Japan). Solvents were degassed by an on-line degasser of the ProStar System. The column used was a RP C18 endcapped Lichrospher column (250×4.6 mm I.D.; 5 μM particle size) was employed (Merck, Darmstadt, Germany), at 30°C. The injection volume was 20 μL and UV detection was performed using UV detector tested using JASCO spectrophotometer (JASCO, Japan) (Figure 10).

Behavioral studies

Male Swiss-Webster mice (Faculty of Pharmacy, Beirut Arab University (BAU), Beirut, Lebanon) were housed for 1 week prior to experimentation. The environment consisted of standard mouse cages with a 12-h light/dark cycle. The temperature was 22 ± 1°C, animals had free access to water and standard laboratory pellets (20% proteins, 5% fats, and 1% multivitamins [20]. All animal care and experiments were done in accordance to Beirut Arab University Institutional Animal Care & Use Committee and with approval of the local ethics commission. All data were tested for significance using one-way ANOVA. A value of $p<0.05$ was considered statistically significant.

Tonic extensor convulsion and toxicity tests

Male mice with an average weight of 18-22 g were used. Reference mice received a single ip injection of 2 mg/kg strychnine to determine the pharmacological end point for tonic extensor convulsions (TECs) and lethality. Control mice were injected ip with 0.2 ml Dimethyl sulfoxide (DMSO) followed 30 minutes later by an ip injection of 2 mg/kg strychnine in DMSO [20]. Test mice were injected ip with serial dilutions of ferutinin, thymoquinone, eugenol or 6-gingerol, and 30 min. later injected ip with 2 mg/kg strychnine. Mice were observed over a period of 1 hour and the time until occurrence of TECs and death was recorded. The first presence of tonus in the hind limbs with stretching was taken as TEC onset, whereas death, preceded by clonic convulsions and tonic seizures, was the pharmacological end point. Another control group of mice was injected with ferutinin, thymoquinone, eugenol or 6-gingerol only; in the highest doses, neither of the test compounds (except strychnine) alone produced convulsions or toxicity.

Determination of Antioxidant Activity with the 2,2'-Diphenyl-1-picrylhydrazyl (DPPH) Radical Scavenging Method

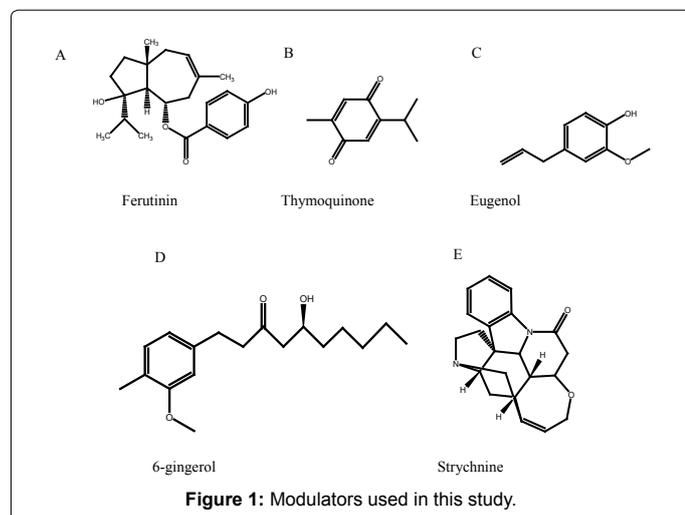The antioxidant activity of ferutinin, thymoquinone, eugenol and

Figure 1: Modulators used in this study.

A Ferutinin

B Thymoquinone

C Eugenol

D 6-gingerol

E Strychnine

6-gingerol were measured in terms of hydrogen donating or radical scavenging ability, using the stable radical DPPH. A methanolic stock solution of ferutinin, thymoquinone, eugenol and 6-gingerol (concentrations of all stock solutions were 5, 25, 50, 100, 150 and 200 µM except thymoquinone 0.5, 1, 2.5, 5, 10 and 20 mM) was put into a cuvette, and 2 mL of 4 mg/ml methanolic solution of DPPH was added. Absorbance measurements commenced immediately. The decrease in absorbance at 517 nm was determined with a JASCO spectrophotometer (JASCO, Japan) at zero time, 20, 40 and 60 minutes for all samples. Methanol was used to zero the spectrophotometer. Absorbance of the DPPH radical without antioxidant, i.e. the control, was measured daily. Special care was taken to minimize the loss of free radical activity of the DPPH radical stock solution. Percent inhibition of the DPPH radical by the samples was calculated according to the formula of Yen & Duh: % inhibition = $[(AC(o) – AA(t)) / AC(o)] * 100$. Where $AC(o)$ is the absorbance of the control at $t = 0$ minute and $AA(t)$ is the absorbance of the antioxidant at $t=1$ h.

Results and Discussions

Quinone and phenolic compounds under investigation, namely, ferutinin, thymoquinone, eugenol and 6-gingerol (Figure 1) have shown positive modulation to GlyRs utilizing preliminary electrophysiological studies using patch-clamp. The protective effects of these compounds against neurodegeneration were mainly investigated in this study using *in vivo* behavioral studies on mice and radical scavenging activity on DPPH.

TEC and lethality test

When administered alone, ferutinin, thymoquinone, eugenol and 6-gingerol at their highest concentrations did not produce any convulsions. None of the tested animals showed any TECs, and test compounds (except strychnine) alone were not otherwise toxic even in highest concentration tested or lethal in any of the test animals. In contrast, an injection of strychnine alone (2 mg/kg ip) was lethal in all tested animals (Figure 2). TEC set in after 7.85 ± 0.35 minutes and death occurred after 8.5 ± 0.42 minutes (n=4). In mice that had been preinjected with either ferutinin (0.1, 0.5 and 1 mg/kg), thymoquinone (1, 3 and 5 mg/kg), eugenol (0.1, 1 and 10 ml/kg) or 6-gingerol (0.1, 1 and 10 mg/kg ip).

In this study, we investigated the *in vivo* effects of ferutinin isolated by column chromatography from seeds of Ferula hermonis (isolated ferutinin) and that of the standard ferutinin (Figure 1A). The purity of the isolated ferutinin was about 96% measured using standard ferutinin calibration curve and spectrophotometer absorbance at 254 nm. In mice that had been preinjected with standard ferutinin (0.1 mg/kg), the toxic effects of strychnine were almost reversed. As, mice featured slight and short reversible TEC without any lethal effect for more than an hour (Figure 2). On the other hand, better effects were shown with isolated ferutinin. As, the mice that had been preinjected with the isolated ferutinin (0.1 mg/kg), the toxic effects of strychnine were completely reversed. The mice were completely protected, as they did not feature neither TEC nor lethal effect for more than an hour (Figure 2). Standard ferutinin at (0.5 mg/kg) significantly counter acted the strychnine toxicity and TEC happened at 9.4 ± 0.9 minutes and death occurred after 10.03 ± 0.8 minutes (n = 4) (Figure 2). While, isolated ferutinin (0.5 mg/kg ip) did not significantly protect the mice from strychnine toxicity. Nevertheless, the highest dose of standard ferutinin (1 mg/kg ip) did not significantly protect the mice from strychnine toxicity. While, the highest dose of isolated ferutinin (1 mg/kg ip) slightly aggravated strychnine toxicity.

This biphasic effect of ferutinin, protective in low doses and aggravating strychnine toxicity in high doses, towards GlyR were observed before in elevation of intracellular calcium in leukemia human Jukart T-cell line [7] and Zn^{2+} ions on the strychnine-sensitive glycine receptor [30]. Therefore, it could be concluded that ferutinin alone, in low doses (0.1 mg/kg ip) protected the mice from strychnine lethality, as expected from GlyR potentiator.

The isolated Tq purity was about 89% measured using standard Tq calibration curve and spectrophotometer absorbance at 254 nm. The toxic effects of strychnine were significantly reduced to about 3 folds (thymoquinone protective effect against TEC = $66.67 \pm 0.50\%$ and against lethality $40.10 \pm 0.90\%$) (Table 1) in the relatively low concentrations (1 mg/kg ip) of thymoquinone (Figure 3). TEC was slightly aggravated, while the toxicity slightly decreased with increasing the concentration of thymoquinone (3 mg/kg ip). Thymoquinone (5 mg/kg ip) showed slight TEC aggravation and non-significant change in toxicity compared to solvent treated group.

The purity of the isolated EUG was about 84% measured using standard ferutinin calibration curve and spectrophotometer absorbance at 215 nm. Strychnine toxic effects were significantly reduced to about 2 folds in the relatively low concentrations (0.1 ml/kg ip) of eugenol (Figure 4) (eugenol protective effect against TEC = 43.66 ± 0.75 %
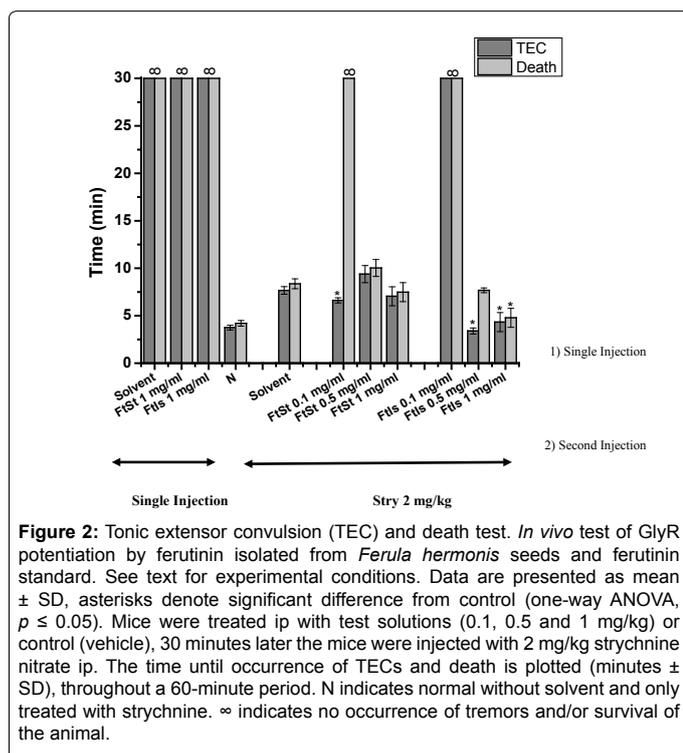

Figure 2: Tonic extensor convulsion (TEC) and death test. *In vivo* test of GlyR potentiation by ferutinin isolated from *Ferula hermonis* seeds and ferutinin standard. See text for experimental conditions. Data are presented as mean ± SD, asterisks denote significant difference from control (one-way ANOVA, $p \le 0.05$). Mice were treated ip with test solutions (0.1, 0.5 and 1 mg/kg) or control (vehicle), 30 minutes later the mice were injected with 2 mg/kg strychnine nitrate ip. The time until occurrence of TECs and death is plotted (minutes ± SD), throughout a 60-minute period. N indicates normal without solvent and only treated with strychnine. ∞ indicates no occurrence of tremors and/or survival of the animal.

	DPPH (IC_{50})	STR-TEC Protective Capacity (*in vivo*) at Lowest Concentration (%)	STR-Lethality Protective Capacity (*in vivo*) at Lowest Concentration (%)
Ft_{Is}	90.41 ± 5.50 µM	99.90 ± 0.10	99.90 ± 0.10
Tq	5.56 ± 0.20 mM	66.67 ± 0.50	40.10 ± 0.90
EUG	41.13 ± 3.50 µM	43.66 ± 0.75	56.04 ± 0.81
6-Gn	28.10 ± 4.10 µM	9.86 ± 0.86	21.97 ± 0.97

Table 1: DPPH antioxidant activity and strychnine (STR) protective (TEC and lethality) capacity (*in vivo*) at the lowest concentration, of isolated ferutinin (FtIs), thymoquinone (Tq), eugenol (EUG) and 6-gingerol (6-Gn).
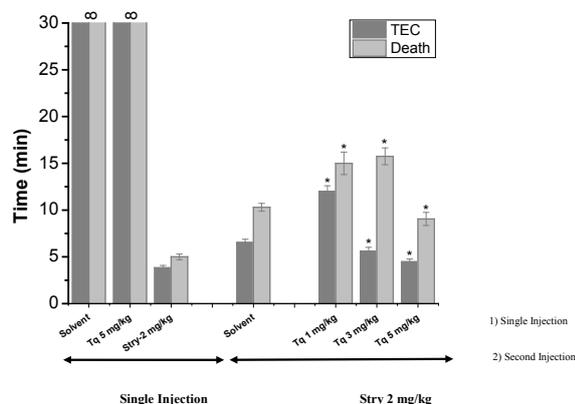
Figure 3. Tonic extensor convulsion (TEC) and death test. *In vivo* test of GlyR inhibition by Black seed oil extracted from *Nigella sativa* seeds (Commercially obtained) and Thymoquinone (Tq). See text for experimental conditions. Data are presented as mean ± SD, "asterisks" denote significant difference from control (one-way ANOVA, $p \leq 0.05$). Mice were treated ip with Tq (1, 3 and 5 mg/kg) or control (vehicle), 30 minutes later the mice were injected with 2 mg/kg strychnine nitrate ip. The time until occurrence of TECs and death is plotted (minutes ± SD), throughout a 60-minute period. ∞ indicates no occurrence of tremors and/or survival of the animal.
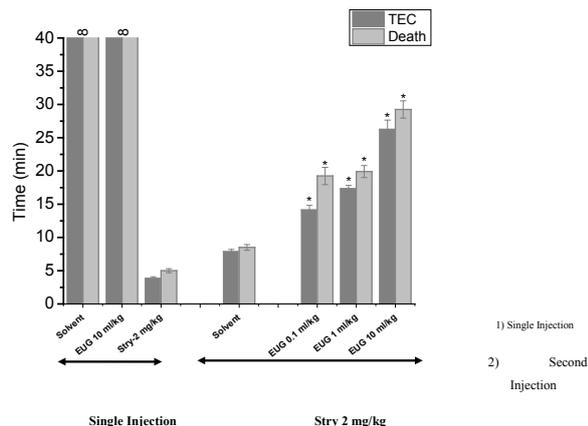

Figure 4: Tonic extensor convulsion (TEC) and death test. *In vivo* test of GlyR potentiation by eugenol (EUG). See text for experimental conditions. Data are presented as mean ± SD, asterisks denote significant difference from control (one-way ANOVA, $p \leq 0.05$). Mice were treated ip with test solutions (0.1, 1 and 10 ml/kg) or control (vehicle), 30 minutes later the mice were injected with 2 mg/kg strychnine nitrate ip. The time until occurrence of TECs and death is plotted (minutes ± SD), throughout a 60-minutes period. ∞ indicates no occurrence of tremors and/or survival of the animal.

and against lethality 56.04 ± 0.81 %) (Table 1). The reduction of TEC and toxicity was slightly increased with increasing the concentration of eugenol (1 ml/kg ip). Eugenol (10 ml/kg ip) showed about 3 folds reduction in TEC and lethality of strychnine.

The isolated 6-Gn purity was about 90% measured using standard 6-Gn calibration curve and spectrophotometer absorbance at 282 nm. 6-Gingerol (0.1 ml/kg ip) showed slight protection against strychnine TEC and toxicity (6-gingerol protective effect against TEC = 9.86 ± 0.86 % and against lethality 21.97 ± 0.97%) (Table 1). TEC was slightly aggravated, while the toxicity not significantly changed with increasing the concentration of 6-gingerol (1 ml/kg ip). 6-Gingerol (10 ml/kg) showed aggravation of strychnine TEC and lethality.

Therefore, as expected form GlyRs modulators, it could be concluded that ferutinin, thymoquinone, eugenol and 6-gingerol when given together with strychnine, in low concentrations reduce strychnine toxicity. It could be concluded that all compounds under investigation could be used as sedatives in low doses as they potentiate the inhibitory GlyRs.

In vivo data showed that ferutinin has a biphasic effect. At low doses (0.1 mg/kg ip *in vivo*), potentiates the GlyRs. While, in relatively high doses (1 mg/kg ip *in vivo*) inhibits the GlyRs. In low concentrations, ferutinin showed to be the most potent protective agent against strychnine toxicity, followed by thymoquinone and eugenol. While, 6-gingerol was the least potent protective agent.

Quinone and phenolic compounds radical scavenging activity on DPPH

In order to fight against neurodegenerative diseases is to improve body antioxidant, the compounds under investigation provided to be good sources for antioxidant potential. A concentration-dependent assay was carried out with ferutinin, thymoquinone, eugenol and 6-gingerol, and the results are presented in Figures 5-9 respectively. These results provide a direct comparison of the antioxidant activity with ascorbic acid (AA). Ferutinin, thymoquinone, eugenol and 6-gingerol possessed significant scavenging activity on the DPPH radical and acted as an antioxidant. The scavenging effect was increased with increasing concentration and reaction time (Figures 5-9).

Ferutinin (Ft) showed a comparatively high scavenging activity at all concentrations (5, 25, 50, 100, 150 and 200 μM). At steady state, when the absorbance of the antioxidant at t = 1 h, IC50 (Ft) = 90.41 ± 5.50 μM (Figure 6).

A comparatively low scavenging activity was shown with thymoquinone (Tq) at all concentrations (0.5, 1, 2.5, 5, 10 and 20 mM). At steady state, when the absorbance of the antioxidant at t = 1 h, IC50 (Tq) = 5.56 ± 0.20 mM (Figure 7). Eugenol (EUG) confirmed to possess
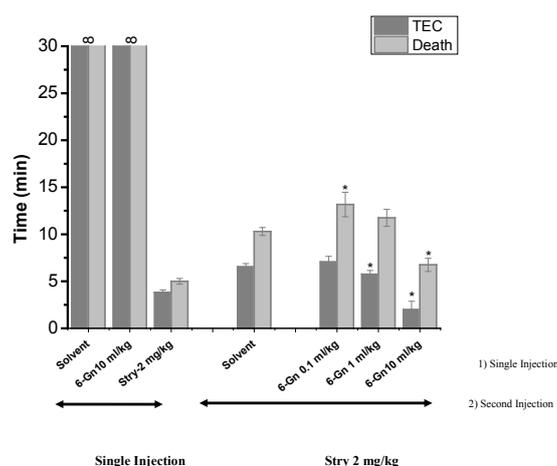

Figure 5: Tonic extensor convulsion (TEC) and death test. *In vivo* test of GlyR potentiation by 6-gingerol (6-Gn). See text for experimental conditions. Data are presented as mean ± SD, asterisks denote significant difference from control (one-way ANOVA, $p \leq 0.05$). Mice were treated ip with test solutions (0.1, 1 and 10 ml/kg) or control (vehicle), 30 minutes later the mice were injected with 2 mg/kg strychnine nitrate ip. The time until occurrence of TECs and death is plotted (minutes ± SD), throughout a 60-minutes period. ∞ indicates no occurrence of tremors and/or survival of the animal.
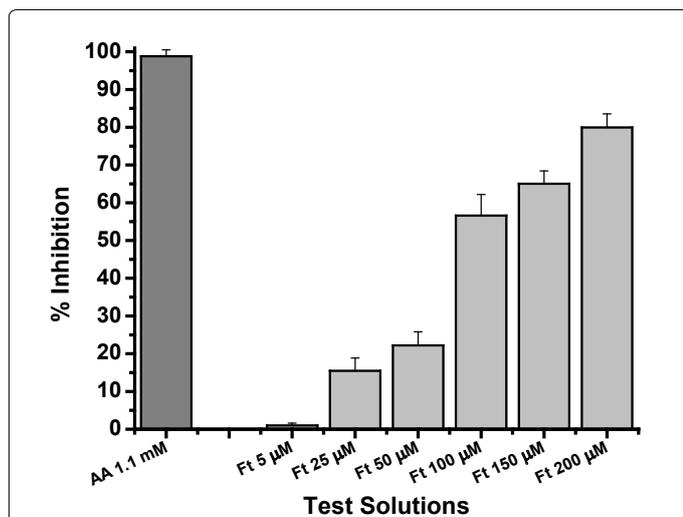
Figure 6: DPPH Antioxidant activity of Ferutinin (Ft) using (5, 25, 50, 100, 150 and 200 µM) concentrations for each. Ascorbic acid (AA) (1.1 mM) is used as positive control. % inhibition = [(AC(o) − AA(t))/ AC(o)]*100. Where AC(o) is the absorbance of the control at t = 0 minute and AA(t) is the absorbance of the antioxidant at t = 1 h. as reported previously (Politeo, 2006). All absorbance were measured at 517 nm. IC50 Ft = 90.41 ± 5.50 µM. Results are (mean ± SD) of three parallel measurements. P < 0.01, when compared to the control.
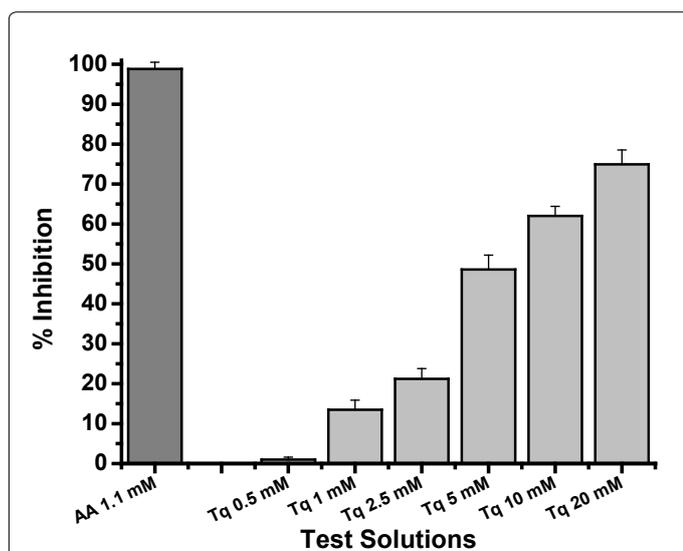

Figure 7: DPPH Antioxidant activity of thymoquinone (Tq) using (0.5, 1, 2.5, 5, 10 and 20 mM) concentrations for each. Ascorbic acid (AA) (1.1 mM) is used as positive control. % inhibition = [(AC(o) − AA(t))/ AC(o)]*100. Where AC(o) is the absorbance of the control at t = 0 minute and AA(t) is the absorbance of the antioxidant at t = 1 h. as reported previously (Politeo, 2006). All absorbance were measured at 517 nm. IC50 Tq = 5.56 ± 0.20 mM. Results are (mean ± SD) of three parallel measurements. P < 0.01, when compared to the control.

comparatively higher scavenging activity at all concentrations (5, 25, 50, 100, 150 and 200 µM). At steady state, when the absorbance of the antioxidant at t = 1 h, IC50 (EUG) = 41.13 ± 3.50 µM (Figure 8). The highest scavenging activity was proved in 6-Gingerol (6-Gn) at all concentrations (5, 25, 50, 100, 150 and 200 µM). At steady state, when the absorbance of the antioxidant at t = 1 h, IC50 (6-Gn) = 28.10 ± 4.10 µM (Figure 9).

Conclusion

We have presented scientific evidence that natural products extracted from neuroactive endogenous medicinal plants, namely, ferutinin, thymoquinone, eugenol and 6-gingerol were verified to be possible modulators to GlyRs; with potential anticonvulsant properties in low doses. These compounds proved to be potential scavengers fighting against neurodegenerative diseases by improving body antioxidant potential. The quinone and phenolic compounds under investigation is suggested to be prophylactic agents acutely administered in response to GlyRs strychnine intoxication and can prevent toxic symptoms, including seizures, convulsions and death.
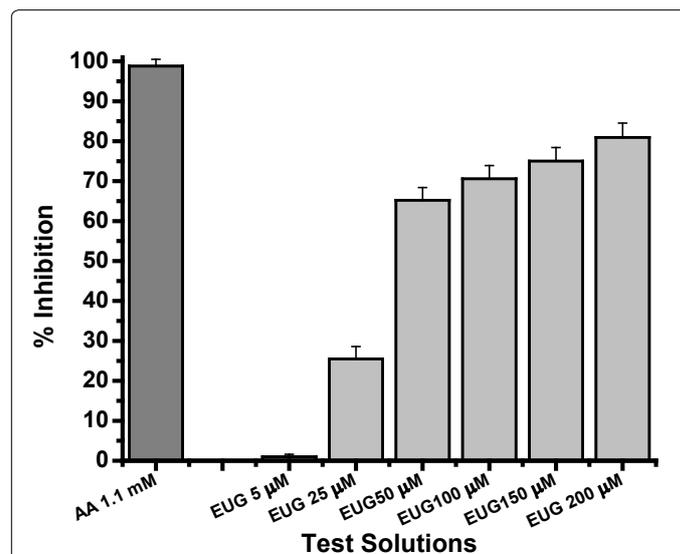

Figure 8: DPPH Antioxidant activity of eugenol (EUG) using (5, 25, 50, 100, 150 and 200 µM) concentrations for each. Ascorbic acid (AA) (1.1 mM) is used as positive control. % inhibition = [(AC(o) − AA(t))/ AC(o)]*100. Where AC(o) is the absorbance of the control at t = 0 minute and AA(t) is the absorbance of the antioxidant at t = 1 h. as reported previously (Politeo, 2006). All absorbance were measured at 517 nm. IC50 EUG = 41.13 ± 3.50 µM. Results are (mean ± SD) of three parallel measurements. P < 0.01, when compared to the control.
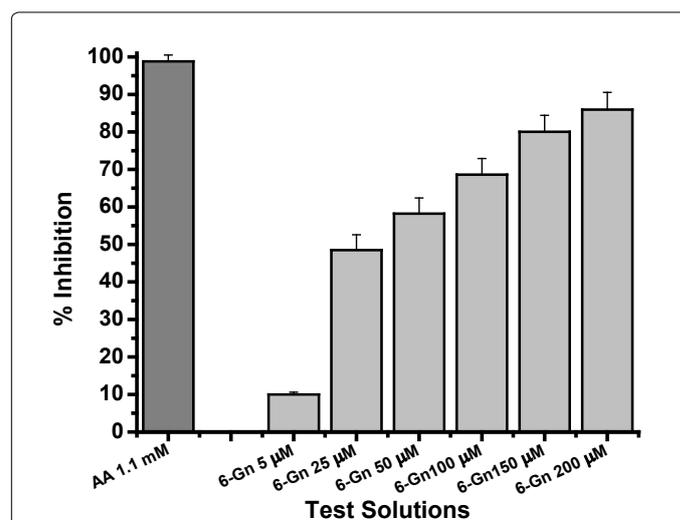

Figure 9: DPPH Antioxidant activity of 6-gingerol (6-Gn) using (5, 25, 50, 100, 150 and 200 µM) concentrations for each. Ascorbic acid (AA) (1.1 mM) is used as positive control. % inhibition = [(AC(o) − AA(t))/ AC(o)]*100. Where AC(o) is the absorbance of the control at t = 0 minute and AA(t) is the absorbance of the antioxidant at t = 1 h. as reported previously (Politeo, 2006). All absorbance were measured at 517 nm. IC50 6-Gn = 28.10 ± 4.10 µM. Results are (mean ± SD) of three parallel measurements. P < 0.01, when compared to the control.
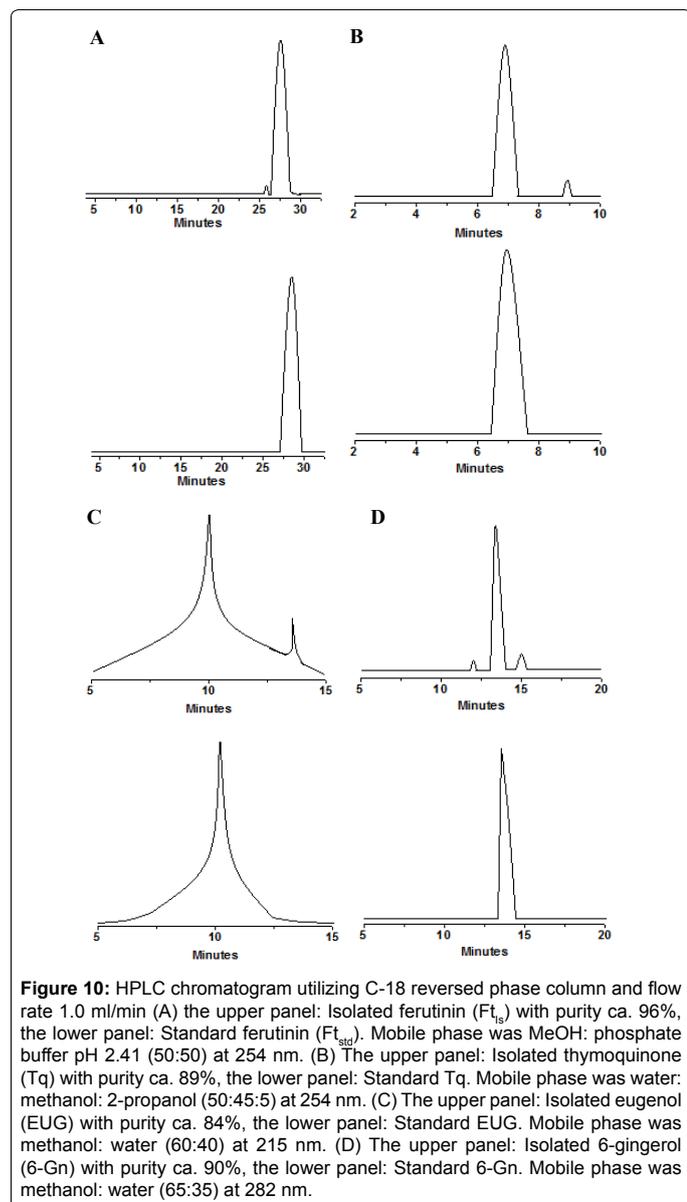
Figure 10: HPLC chromatogram utilizing C-18 reversed phase column and flow rate 1.0 ml/min (A) the upper panel: Isolated ferutinin (Ft$_{is}$) with purity ca. 96%, the lower panel: Standard ferutinin (Ft$_{std}$). Mobile phase was MeOH: phosphate buffer pH 2.41 (50:50) at 254 nm. (B) The upper panel: Isolated thymoquinone (Tq) with purity ca. 89%, the lower panel: Standard Tq. Mobile phase was water: methanol: 2-propanol (50:45:5) at 254 nm. (C) The upper panel: Isolated eugenol (EUG) with purity ca. 84%, the lower panel: Standard EUG. Mobile phase was methanol: water (60:40) at 215 nm. (D) The upper panel: Isolated 6-gingerol (6-Gn) with purity ca. 90%, the lower panel: Standard 6-Gn. Mobile phase was methanol: water (65:35) at 282 nm.

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