Cholinesterase and lipoxygenase inhibition of whole plant *Withania somnifera*

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To discover new therapeutically effective and safe cholinesterase and lipoxygenase inhibitors, crude methanol extract and subsequent solvent fractions of *Withania somnifera* Dunal were screened for their inhibitory profile. Results revealed marked overall inhibition. Against acetylcholinesterase, the IC₅₀ values were in the range of 69 to 111 µg/ml, except hexane fraction. Similarly, profound lipoxygenase attenuation was observed (IC₅₀: 76 to 132 µg/ml) for extracts with the exception of hexane fraction. It is notable that among the extracts, chloroform fraction was the most dominant inhibitor of the tested enzymes. This study indeed provides a strong scientific rationale for the folk use of the plant for the loss of memory and as anti-inflammatory.

Key words: *Withania somnifera*, Solanaceae, cholinesterase and lipoxygenase inhibition.

INTRODUCTION

Around the globe, medicinal plants have provided humanity with numerous therapeutic agents. Infact, medicinal plants is considered as the most dynamic and oldest form of medication (Qadrie et al., 2009; Nisar et al., 2010; Khan et al., 2011a; Ghasi et al., 2011). Even today, despite the outstanding development in the field of synthetic drugs development, plant based drugs have sound market value (Saeed et al., 2010a; Khan et al., 2011b; Saeed et al., 2011) most probably due to safety reasons. For that reason, it is curial to evaluate medicinal plants on scientific grounds for new drugs development.

*Withania somnifera* Dunal (Family: Solanaceae, Vernacular: Sanskrit: Aswagandha; Telugu: Panneru; Pashto: Khotilal; Trade name: Aswagandha) is an important drug in the ancient system of Ayurveda used for curing of a variety of ailments and is widely distributed in Pakistan, India, Sri Lanka, Mediterranean regions, Canaries, South Africa, Iraq, Iran, Syria and Turkey (Thakur et al., 1989). The roots are used in senile debility, rheumatism, nervous exhaustion, loss of memory, loss of muscular energy and spermatorrhoea. The decoction of the root boiled with milk and vegetable oil is recommended for curing sterility in women (Kirtika and Basu, 1991). Withanolides with antitumor activity have been isolated (Singh et al., 2001; Ali et al., 1997). The methanol and hexane extracts of the aerial parts of *W. somnifera* showed significant antimicrobial activity against Gram positive bacteria (Arora et al., 2004).

The anti-inflammatory activity, protective effect against CCl₄-induced hepatotoxicity and neuro-protective effect in Parkinson's disease of *W. somnifera* have also been characterized (Ahmad et al., 2005). The plant was found to be active on animal model of Alzheimer's disease and perturbed central cholinergic markers of cognition in rats and showed usefulness in nervous breakdown probably due to anti-cholinesterase activity (Vinutha et al., 2007). Phytochemically, *W. somnifera* mainly contains withanolides (basically steroidal lactones), alkaloids and other minor chemical constituents (Misra et al., 2005).

Keeping in view the potential of the plant in the...
treatment of different diseases, the current study was designed to evaluate the inhibitory profile of plant extracts against various enzymes.

MATERIALS AND METHODS

Plant

The whole plant of W. somnifera Dunal was collected from the tribal area, Khyber agency of Khyber Pakhtoonkhawa, Pakistan and was identified by Mr. Shahid Farooq of the botany section of Pakistan Counsel of Scientific and Industrial Research (PCSIR) laboratories complex, Peshawar. A voucher specimen no 9741 (PES) was deposited in the herbarium of PCSIR Peshawar.

Extraction and fractionation

The shade-dried whole plant material (20 kg) was chopped and extracted thrice with methanol (40 L) with occasional shaking, at room temperature. After 15 days, the methanol soluble materials were filtered off. The filtrate was concentrated under vacuum at room temperature. After 15 days, the methanol soluble materials were filtered off. The filtrate was concentrated under vacuum at low temperature. After 15 days, the methanol soluble materials were filtered off. The filtrate was concentrated under vacuum at low temperature.

In-vitro cholinesterase inhibition assay

The standard operational assay was employed to determine the AChE and BChE inhibition activities (Khan et al., 2010b). Enzymes and reagents such as electric-eel AChE (EC 3.1.1.7), horse-serum BChE (EC 3.1.1.8), acetylthiocholine iodide, butyrylthiocholine chloride, 5, 5´-dithiobis [2-nitrobenzoic acid] (DTNB) and galanthamine were purchased from Sigma (St. Louis, MO, USA). All other chemicals were of molecular biology grade. Acetylthiocholine iodide and butyrylthiocholine chloride were used as the substrates to assay AChE and BChE activities, respectively. The reaction mixture contained 150 µl of sodium phosphate buffer (100 mM) (pH 8.0), 10 µl of DTNB, 10 µl (0.2 mM) of test-compound solution and 20 µl of AChE or BChE solution, which were mixed and incubated for 15 min at 25°C. The reaction was then initiated with the addition of 10 µl acetylthiocholine or butyrylthiocholine, respectively. The hydrolysis of acetylthiocholine and butyrylthiocholine were monitored by the formation of yellow 5-thio-2-nitrobenzoate anion resulting from the reaction of DTNB with thiococholine, catalyzed by acetylthiocholine, and butyrylthiocholine, respectively at a wavelength of 412 nm (15 min). Test compounds and the positive control (galanthamine) were dissolved in ethanol. As the extinction coefficient of the yellow anion is known, the rate of enzymatic reaction was finally determined by applying the Ellman equation.

In-vitro lipoxygenase inhibition assay

Lipoxygenase inhibiting activity was conveniently measured by slightly modifying the spectrometric method developed previously (Khan et al., 2009; Khan et al., 2011b). Lipoxygenase (1,13.11.12) type I-B and linoleic acid were purchased from Sigma (St. Louis, MO) and were used without further purification. All other chemicals were of analytical grade. 160 ml of sodium phosphate buffer, 0.1 mM (pH 7.0), 10 ml of the sample solution and 20 ml of lipoxygenase solution were mixed and incubated for 5 min at 25°C. The reaction was initiated by the addition of 10 µl linoleic acid solution substrate and the absorption change with the formation of (9Z, 11E)-13S-13-hydroperoxyoctadeca-9, and 11-dienoate followed for 10 min. The test sample and the control were dissolved in 50% ethanol. All the reactions were performed in triplicate. The IC50 values (concentrations of sample causing 50% reduction in activity relative to the control) were calculated using the EZ-Fit Enzymes kinetics programme.

RESULTS

In-vitro AChE and BChE inhibition assay

The result of AChE inhibition revealed that the extract exhibited prominent inhibition as shown in Figure 1 and Table 1. However, insignificant activity was observed against BChE. The chloroform fraction was most potent (IC50: 69 µg/ml) inhibitor followed by crude extract (IC50:76 µg/ml) and ethyl acetate (IC50:78 µg/ml).

In-vitro lipoxygenase inhibition assay

The results of lipoxygenase inhibition are presented in Figure 2 and Table 2. The crude extract and subsequent fractions of W. somnifera Dunal showed significant inhibitor activity. The chloroform fraction of the plant was the most potent lipoxygenase inhibitor with IC50 as 76 µg/ml. This trend of lipoxygenase inhibition was followed.

Figure 1. Cholinergic inhibition of the crude extract and fractions of whole plant of W. somnifera. Values are the mean ± SEM of three assays.
Table 1. IC$_{50}$ values of the crude extract and various fractions of the whole plant of *W. somnifera* against acetylcholinesterase.

<table>
<thead>
<tr>
<th>Drug/Fractions</th>
<th>IC$_{50}$ ± SEM (µg/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Acetylcholinesterase</td>
<td></td>
</tr>
<tr>
<td>Crude</td>
<td>76 ± 0.16</td>
</tr>
<tr>
<td>Hexane</td>
<td>-</td>
</tr>
<tr>
<td>Chloroform</td>
<td>69 ± 0.20</td>
</tr>
<tr>
<td>Ethyl acetate</td>
<td>78 ± 0.24</td>
</tr>
<tr>
<td>Butanol</td>
<td>97 ± 0.23</td>
</tr>
<tr>
<td>Water</td>
<td>111 ± 0.14</td>
</tr>
<tr>
<td>Galanthamine</td>
<td>22.6 ± 0.09</td>
</tr>
</tbody>
</table>

IC$_{50}$ values are the mean ± SEM of three assays.

Table 2. IC$_{50}$ values of the crude extract and various fractions of the whole plant of *W. somnifera* against lipoxygenase.

<table>
<thead>
<tr>
<th>Drug/Fractions</th>
<th>IC$_{50}$ ± SEM (µg/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lipoxygenase</td>
<td></td>
</tr>
<tr>
<td>Crude</td>
<td>81 ± 0.13</td>
</tr>
<tr>
<td>Hexane</td>
<td>-</td>
</tr>
<tr>
<td>Chloroform</td>
<td>76 ± 0.36</td>
</tr>
<tr>
<td>Ethyl acetate</td>
<td>97 ± 0.28</td>
</tr>
<tr>
<td>Butanol</td>
<td>128 ± 0.19</td>
</tr>
<tr>
<td>Water</td>
<td>132 ± 0.12</td>
</tr>
<tr>
<td>Baicalein</td>
<td>22.6 ± 0.09</td>
</tr>
</tbody>
</table>

IC$_{50}$ values are the mean ± SEM of three assays.

DISCUSSION

In terms of plants biodiversity, Pakistan possesses a dominant status among the developing countries most probably due to varied climatic and edaphic factors. Around the country, it has been speculated that approximately 6000 taxa of flowering plants can be found. On the basis of ethnobotanical investigations, approximately 600 to 1000 plants have been found to have one or the other medicinal properties and are used in the management of different pathological conditions. In the local drug markets, approximately 350 to 400 species are traded and used by various manufacturers in the formulation of herbal drugs (Saeed et al., 2010b, 2010c). Keeping in mind the tremendous potential of Pakistani medicinal plants, the present study was therefore, designed to evaluate the enzyme inhibitory profile of the whole plant of *W. somnifera*.

Alzheimer's disease (AD) is a disorder coupled with progressive degeneration of memory and cognitive function. Cholinesterase inhibitors are the recommended drugs for treating patients with mild to severe AD (Sancheti et al., 2010). The cholinergic theory assumes that, memory impairment in patient with Alzheimer's disease occurs from a shortfall of cholinergic activity in the brain. Acetylcholinesterase inhibitors can reinstate the amount of acetylcholine by inhibiting AChE. Over the years, medicinal plants have offered components with decent activity (Khan et al., 2009) and could be a potential source. Lipoxygenases (LOXs) are responsible for the metabolism of the fatty acids (FA) and their metabolites cause inflammatory responses in the body. They also play significant role in cancer cell growth, metastasis, invasiveness, cell survival and induction of tumor necrosis factor (TNF) (Hallahan et al., 1994; Chan, 1995). The 12/15-lipoxygenase has role in inflammatory diseases including atherosclerosis, cancer, osteoporosis, angiotension II-dependent hypertension and diabetes (Hartmut et al., 2006; Wube et al., 2006). Inhibition of the 5-LOX pathway has a chemopreventive effect in animal lung carcinogenesis and blocks the oxidation of several potent carcinogens (Moody et al., 1998). Lipoxygenases are therefore potential targets for the rational design and discovery of mechanism-based inhibitors. As the plant extracts exhibited profound inhibitory effect on lipoxygenase, it is therefore, assumed that the plant can be a useful natural source of lipoxygenase inhibitors.

Conclusion

Conclusively, our findings provide scientific background to the traditional uses of the plant in the treatment of memory impairment and rheumatic conditions. Moreover, the isolation of secondary metabolites with activity against said enzymes will further augment the uses of the plant. Further detailed studies are required for the
discovery of new lead compounds with prominent efficacy and safety.

REFERENCES


