Anxiolytic and Sedative Activities of the Essential Oil of the Fresh Young Shoot of *Asparagus officinalis* L. in Mice

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**Abstract**

**Background:** Asparagus officinalis L. is an edible plant that serve as a vegetable and medicine in different parts of the world, including CNS-related ailments.

**Objective:** To evaluate the anxiolytic and sedative activities *Asparagus officinalis* L. fresh young shoot essential oil of *Asparagus officinalis* L. (EOAO) in mice.

**Method:** The essential oil was obtained through hydro distillation using clevenger-type apparatus. The effect of EOAO (6.25, 12.5 and 25 mg/kg, i.p., n=6) on anxiety was evaluated using the elevated plus maze and hole board test. The sedative effect of the EOAO (50, 100 and 150 mg/kg, i.p., n=6) was evaluated using ketamine-induced hypnosis (100 mg/kg, i.p.).

**Results:** The EOAO (6.25, 12.5 and 25 mg/kg i.p.) and diazepam 1 mg/kg i.p., increased significantly (p < 0.01) the time spent in the open arms of the elevated plus maze (EPM) compared to the vehicle. There was significant increase in head dips (p < 0.01) at 6.25, 12.5 and 25 mg/kg i.p. of the administered oil. The EOAO at 50, 100 and 150 mg/kg, i.p. and diazepam (2 mg/kg, i.p.) significantly (p < 0.01 – 0.001) reduced the sleep latency when compared to the vehicle. At 50, 100 and 150 mg/kg, i.p. the EOAO and diazepam (2 mg/kg, i.p.) significantly (p < 0.01 - 0.001) prolonged the total sleeping time when compared to the vehicle.

**Conclusion:** The study concluded that *Asparagus officinalis* L. essential oil possesses anxiolytic and sedative activities.

**Keywords:** *Asparagus officinalis*, Anxiolytic, Sedative properties
INTRODUCTION

The use of medicinal plants to treat a wide range of ailments has expanded in developing countries (Theophine et al., 2014). Herbal medications are widely perceived to be safer, have fewer adverse effects and are readily available locally than manufactured pharmaceuticals (Cohen and Ernst, 2010; Mohd et al., 2019). For over 2,000 years, Asparagus has been grown as a medicinal herb and also serves as a vegetable. In ethnomedicine, both the roots and the shoots are of importance. The different species of Asparagus are widely evaluated for their nutritional, biological and pharmacological actions (Pegiou et al., 2020). Asparagus species have their natural distribution across Asia, Africa and Europe (Thakur and Sharma, 2015).

In Ayurveda, Asparagus is used as a nerve tonic, famous in the ancient Greek, Persian, and Chinese traditional medicine, and possesses neuroprotective, anti-diabetic, antioxidant, adaptogenic, nootropic activity, preventing oxidative neuronal damage (Rajasekhar et al., 2019; Majumdar et al., 2021), anti-inflammatory and as a sedative agent (Atanasov et al., 2015). In India, the roots of Asparagus officinalis L. are used to promote improvement both in mental and physical health (Dahanukar et al., 2000; Orjha et al., 2010). Despite the studies done on the plant A. officinalis L, there is scanty information on the biological activities of the essential oil of the fresh shoot (aerial part) of the plant. Essential oils have been reported to demonstrate several interesting central nervous system activities including behavioural, sedative, anxiolytic, and anticonvulsant activities (Oyemitan et al., 2013). Preliminary studies on the essential oil of A. officinalis L. showed significant CNS depressant effects at higher doses tested (Unpublished result). Hence, the focus of this study included the evaluation of the essential oil of this plant species for anxiolytic and sedative activities. This is to establish the scientific basis for the ethnomedicinal uses of the plant and serving as lead in the discovery of new moieties for drug discovery and development.

METHODOLOGY

Plant collection, identification, authentication and preparation

The plant, A. officinalis L. was collected at Kajola-Ajile Zone 4, Ede Road, Ile-Ife, Nigeria. It was identified and authenticated by Mr I.I. Ogunlowo, the herbarium officer of the Pharmacognosy Department, Faculty of Pharmacy, Obafemi Awolowo University, Ile-Ife, and herbarium specimen number FPI2348 was issued. The volatile fraction of A. officinalis L. fresh young shoot was obtained by a process of hydro distillation for about 4 h using a Clevenger-type apparatus, at the Postgraduate Toxicology Laboratory, Department of Biochemistry and Molecular Biology, Faculty of Science, Obafemi Awolowo University, Ile-Ife, Nigeria. Pale yellow oil produced was collected after cooling and stored in the refrigerator (4°C) to freeze any water content and obtain the pure oil. The Essential oil of A. officinalis L. was weighed and emulsified with Tween - 80 and diluted with distilled water to the required concentration.

Laboratory animals

Adult male and female albino mice (18 - 25 g) obtained from the Animal House, Department of Pharmacology, Faculty of Pharmacy, OAU, Ile-Ife were used for the study. They were supplied with food and water ad libitum. The formulated essential oil and test drugs were administered intraperitoneally (i.p.). The animal experiment was carried out as approved by the Committee on Animal Use and Care of the OAU, Ile-Ife, Nigeria.

Anxiolytic activity assessment on hole board

Thirty mice were allotted into groups of six mice each. Group I received 5% Tween – 80 (0.1 ml/10 g) as negative control intraperitoneally (i.p.), Group II, III and IV were administered with different doses – 6.25 mg/kg i.p., 12.5 mg/kg i.p. and 25 mg/kg i.p. of essential oil of Asparagus officinalis L. (EOAO) respectively via intraperitoneal (i.p.) route and group V received Diazepam (1mg/kg i.p.) to serve as the positive control. The hole board is a flat space (field) of 25 cm², with 16 holes, each 3 cm in diameter. After 30 min pretreatment, each mouse was gently placed at the center of the hole board from the home cages. The number of head-poking (head dips) made by each mouse was recorded. For the assessment, an increase in exploratory activity (number of head dips) indicates...
an anxiolytic effect, while a decrease shows an anxiogenic effect (Oyemitan et al., 2016).

**Anxiolytic activity assessment on elevated plus maze**

The elevated plus-maze apparatus is made of wooden material, consisting of two open and two closed arms across each other, respectively. Thirty mice were randomly allotted into five groups (n=6); Group I - 5% Tween-80 (0.1 ml/g), Groups II-IV - essential oil of Asparagus officinalis L. (6.25 mg/kg i.p., 12.5 mg/kg i.p. and 25 mg/kg i.p.) and Group V - Diazepam (1mg/kg i.p.) doses were administered intraperitoneally (i.p.). After 30 min pretreatment of the mice with all the test agents, each mouse was tested on the EPM to assess the number of entries in the open and closed arms and the time spent in each of the arms. The index of open arm avoidance was then calculated. Index of open arm avoidance (IOAA) = 100 - [ (% time spent in open arms + % entries into open arms)/2] (Trullas and skolnick, 1993; Oyemitan et al., 2013).

**Sedative activity evaluation using ketamine-induced hypnosis**

The test was carried out using the method described by Mimura et al., 1990; Oyemitan et al., 2016, to determine the effects of essential oil of A. Officinalis L. (EOAO) on latency and prolongation of sleep time induced by ketamine. Thirty mice were randomly allotted into five groups (n=6). Group I was administered with 5% Tween – 80 (negative control, 0.1 ml/g, i.p.), II–IV were injected with various doses (50, 100, and 150 mg/kg, i.p.) of EOAO respectively. Group V was injected with 2 mg/kg, i.p. of diazepam (positive control). After 30 min of pretreatment of the mice with all test materials: (5% Tween and diazepam), ketamine (100 mg/kg, i.p.) was administered. The time interval between ketamine administration and loss of righting reflex was considered as sleep latency (SL), while the time from the loss to the regaining of righting reflex as total sleep time (TST) (Rabbani et al., 2003).

**Statistical analysis**

The results were expressed as Mean ± SEM and analysed using one-way analysis of variance (ANOVA) followed by a post hoc test using Dunnett’s comparison test. The level of significance was set at 95% confidence interval (p < 0.05) for all treatments carried out compared to control groups. Graph pad prism, version 5.0 (UK) was used for the analysis.

**RESULTS**

**Effect of Asparagus officinalis L. essential oil on hole board**

There was an increase in head dips compared to the vehicle when 6.25 mg/kg i.p., 12.5 mg/kg i.p. and 25 mg/kg i.p. of the EOAO were administered. However, there was statistical significance (p < 0.01) only at 12.5 mg/kg i.p. of EOAO compared to positive control, diazepam 1 mg/kg i.p. (figure 1).

**Effect of Asparagus officinalis L. essential oil on elevated plus maze**

Effect of Asparagus officinalis L. essential oil on percentage time spent in open and closed arms /300 s on EPM

At lower doses of EOAO tested (6.25 mg/kg i.p., 12.5 mg/kg i.p. and 25 mg/kg i.p.) and diazepam 1 mg/kg i.p., the time spent in the open arms of the EPM compared to the vehicle increased significantly (p < 0.01). The various mean percentage times spent in the open arm of the EPM were found to be 60.28, 46.44, 49.50 and 54.61 for the EOAO (6.25 mg/kg i.p., 12.5 mg/kg i.p. and 25 mg/kg i.p.) and diazepam (1 mg/kg, i.p.) respectively. (Table 1).

**Effect of Asparagus officinalis L. essential oil on percentage entries into open and closed arm /300 s on EPM**

The EOAO (6.25 mg/kg i.p., 12.5 mg/kg i.p. and 25 mg/kg i.p.) showed no significant difference (except the positive control group) in the number of entries into the open arms of the EPM compared to the vehicle. The various mean percentage number of entries into the open arm of the EPM were found to be 50.00, 52.23, 52.23, 50.93 and 50.00 for the vehicle (0.1 ml/10 g), EOAO (6.25 mg/kg i.p., 12.5 mg/kg i.p. and 25 mg/kg i.p.) and diazepam (1 mg/kg i.p.) respectively (Table 2).

**Index of Open Arm Avoidance (IOAA)**

100 - [(% time spent in open arms + % entries into open arms)/2] For vehicle (0.1 ml/10 g) = 100 - [(10.67 + 50.00)/2] = 69.67 EOAO (6.25 mg/kg i.p.) = 100 - [(60.28 + 52.23)/2] = 43.75 EOAO (12.5 mg/kg i.p.) = 100 - [(46.44 + 52.23)/2] = 50.67 EOAO (25 mg/kg i.p.) = 100 - [(49.50 + 50.93)/2] = 49.79
DZM (1 mg/kg i.p.) = 100 - [((54.61 + 50.00) / 2] = 47.70 (Table 3)

Effect of *Asparagus officinalis* L. essential oil on ketamine-induced hypnosis
Effects of *Asparagus officinalis* L. essential oil on ketamine-induced sleep latency (SL) in mice
The EOAO at 50, 100 and 150 mg/kg, i.p. and diazepam (2 mg/kg, i.p.) significantly (p < 0.01 – 0.001) reduced the sleep latency when compared to the vehicle (5% Tween 80 0.1 ml/10g i.p.) as shown in Figure 2A.

Effects of EOAO on ketamine-induced total sleeping time (TST) in mice
The EOAO (50, 100 and 150 mg/kg, i.p.) and diazepam (2 mg/kg, i.p.) significantly prolonged the total sleeping time (p < 0.05 - 0.001) when compared to the vehicle as shown in Figure 2B.

**Figure 1: Effect of EOAO on head dip**

Bars represent mean values with error bars (n=6). VEH, EOAO and DZP represent vehicle (5% Tween 80), *Asparagus officinalis* L. essential oil and diazepam respectively.

**p < 0.01 statistically significant compared to the vehicle (ANOVA, Dunnett’s test)

##p < 0.01 statistically significant compared to the diazepam (ANOVA, Dunnett’s test)
Table 1: Effect of EOAO on the percentage time spent in open and closed arms/300 s on the EPM

<table>
<thead>
<tr>
<th>Treatment groups (n=6)</th>
<th>VEH (0.1 ml/10 g, i.p.)</th>
<th>EOAO (6.25 mg/kg, i.p.)</th>
<th>EOAO (12.5 mg/kg, i.p.)</th>
<th>EOAO (25 mg/kg, i.p.)</th>
<th>DZP (1 mg/kg, i.p.)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>EOA</td>
<td>ECA</td>
<td>EOA</td>
<td>ECA</td>
<td>EOA</td>
</tr>
<tr>
<td>Mean ± SEM</td>
<td>2.33 ± 0.82</td>
<td>5.83 ± 3.55</td>
<td>4.17 ± 3.83</td>
<td>4.67 ± 4.50</td>
<td>6.00 ± 3.10</td>
</tr>
<tr>
<td>Percentage entry (%)</td>
<td>50.00</td>
<td>52.23</td>
<td>52.13</td>
<td>50.93</td>
<td>50.00</td>
</tr>
</tbody>
</table>

VEH, EOAO, DZP, EOA and ECA represent vehicle, *Asparagus officinalis* L. essential oil, diazepam, entries into open arm and entries into closed arm respectively.
*p < 0.05 statistically significant compared to the vehicle (ANOVA, Dunnett’s test).

Table 2: Effect of EOAO on percentage entries into open and closed arm/300 s on EPM

<table>
<thead>
<tr>
<th>Treatment groups (n=6)</th>
<th>VEH (0.1 ml/10 g, i.p.)</th>
<th>EOAO (6.25 mg/kg, i.p.)</th>
<th>EOAO (12.5 mg/kg, i.p.)</th>
<th>EOAO (25 mg/kg, i.p.)</th>
<th>DZP (1 mg/kg, i.p.)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>TSOA</td>
<td>TSCA</td>
<td>TSOA</td>
<td>TSCA</td>
<td>TSOA</td>
</tr>
<tr>
<td>Mean ± SEM</td>
<td>32.00 ± 15.71</td>
<td>180.83 ± 67.15</td>
<td>139.33 ± 53.44</td>
<td>148.50 ± 15.08</td>
<td>163.83 ± 47.64</td>
</tr>
<tr>
<td>Percentage time spent (%)</td>
<td>10.67 ± 5.32</td>
<td>60.28 ± 39.72</td>
<td>46.44 ± 53.56</td>
<td>49.50 ± 50.50</td>
<td>54.61 ± 45.391</td>
</tr>
</tbody>
</table>

VEH, EOAO, DZP, TSOA and TSCA represent vehicle, *Asparagus officinalis* L. essential oil, diazepam, time spent in open arm and time spent in closed arm respectively.
**p < 0.01 statistically significant compared to the vehicle (ANOVA, Dunnett’s test).
Table 3: Effect of EOAO on index of open arms avoidance (IOAA) on the EPM

<table>
<thead>
<tr>
<th>Treatment groups (n=6)</th>
<th>VEH (0.1 ml/10 g, i.p.)</th>
<th>EOAO (6.25 mg/kg, i.p.)</th>
<th>EOAO (12.5 mg/kg, i.p.)</th>
<th>EOAO (25 mg/kg, i.p.)</th>
<th>DZP (1 mg/kg, i.p.)</th>
</tr>
</thead>
<tbody>
<tr>
<td>% TSOA</td>
<td>10.67</td>
<td>60.28</td>
<td>46.44</td>
<td>49.50</td>
<td>54.61</td>
</tr>
<tr>
<td>% EOA</td>
<td>50.00</td>
<td>52.23</td>
<td>52.13</td>
<td>50.93</td>
<td>50.00</td>
</tr>
<tr>
<td>IOAA</td>
<td>69.67</td>
<td>43.70</td>
<td>50.72</td>
<td>49.79</td>
<td>47.70</td>
</tr>
</tbody>
</table>

IOAA = 100 - [(% time spent in open arms + % entries into open arms)/2]  
IOAA, EOAO, % TSOA, % EOA and DZP represent, index of open arms avoidance, Asparagus officinalis L. essential oil, % time spent in open arms, % entries into open arms and diazepam respectively.  
The IOAA value for the EOAO is at least 10 points < IOAA value for vehicle.

Figure 2: Effect of EOAO on sleep latency (A) and Total sleeping time (B)

Bars represent mean values with error bars (n=6). VEH, EOAO and DZP represent vehicle (5% Tween 80), Asparagus officinalis L. essential oil and diazepam respectively.  
***p < 0.001, **p < 0.01, *p < 0.05 statistically significant compared to the vehicle (ANOVA, Dunnett’s test)
DISCUSSION

The major effect of the essential oil observed from the preliminary study revealed a biphasic effect on the CNS. Acute toxicity testing is an initial test of determining the toxicity profile of unknown substances, such as medicinal plant extracts, essential oils, isolates, and other natural products. The median lethal dose which is an index of acute toxicity that provides preliminary information on acute toxicity was done according to Lorke’s method (Lorke, 1983) (Unpublished result).

The sedative activity of the oil was observed during the median lethal dose determination. Some agents with sedative property like benzodiazepines, barbiturates, and also some researched medicinal plants have been found to elicit an anxiolytic activity at lower doses (Abubakar and Haque, 2019). Hence, EOAO was subjected to anxiolytic activity test at lower doses (6.25 mg/kg i.p., 12.5 mg/kg i.p., and 25 mg/kg i.p.) using two different models (hole board and elevated plus maze).

The hole board experiment evaluates the anxiolytic or anxiogenic activity of new drugs in rodents (Lister, 1990). The head dips made by rodents are inversely proportional to neophobia (Brown and Nemes, 2008). An increase in the number of head dips into the holes on the board depicts a reduced anxiety (Oyemitan et al., 2017). There was a significant (p < 0.01) increase in head dips compared to the vehicle when 6.25 mg/kg i.p., 12.5 mg/kg i.p., and 25 mg/kg of EOAO were administered. This significant increase in head dips, therefore, shows the anxiolytic effect of the tested lower doses of EOAO.

The essential oil at all the doses tested and diazepam 1 mg/kg p.o., increased significantly (p < 0.01) the time spent in the open arms of the EPM compared to the vehicle. There was however no significant difference (except the positive control group) in the number of entries into the open arms of the EPM compared to the vehicle. Generally, anxiolytic drugs cause an increment in the time spent in the open arm and also the number of entries into the open arms of the EPM (Akanmu et al., 2011). The index of open arm avoidance (IOAA) gives a measure of anxiety (Trullas and Skolnick, 1993). When the IOAA of a test substance is at least 10 points less than that of the vehicle, it is indicative of the anxiolytic activity of the test substance but when the IOAA of the test substance is at least 10 points greater than that of the vehicle, it shows the test substance is anxiogenic. The IOAA across the test groups was at least 10 points less than that of the vehicle and this suggests anxiolytic activity (Akanmu et al., 2011).

The sedative activity was evaluated using ketamine. Ketamine is an antagonist of the NMDA receptor, an excitatory receptor that plays a role in seizures and is known to cause hypnosis also. Results from the effect of the oil on ketamine-induced sleeping time showed that EOAO at 50, 100 and 150 mg/kg, i.p., and diazepam (2 mg/kg, i.p.) significantly (p < 0.01 – 0.001) reduced the sleep latency when compared to the vehicle (5% Tween 80 0.1 ml/10g i.p.). The total sleeping time was also significantly (p < 0.05 - 0.001) prolonged compared to the vehicle when EOAO (50, 100 and 150 mg/kg, i.p.) and diazepam (2 mg/kg, i.p.) were administered.

In regulation of sleep and wakefulness various neurotransmitters and endogenous molecules are involved. The sleep-promoting neurons located in the anterior hypothalamus release gamma-amino butyric acid (GABA) to suppress the activity of wake-inducing areas of the brain (Datta, 2010). Ketamine has been established to exhibit agonist properties on GABA receptors (Henschel et al., 2008) hence, it suggests a possible involvement of the GABAergic pathway in the eliciting of the sedative activity of the oil (Sivam et al., 2004).

Some medicinal plants act via the GABAergic system to induce their sedative/hypnotic effect (Nogueira et al., 2000) or inhibit the effect of glutamate (an excitatory neurotransmitter) via the blockade of glutamate receptors including: N-methyl-Daspartate (NMDA), AMPA, kainate, glycine or metabotropic receptors (Trevor and Way, 2009; Akuegbe et al., 2019).

Studies carried out previously have reported that the presence of some phytochemical compounds including terpenes, flavonoids, and saponins are responsible for the observed hypnotic activity of medicinal plants (Jiang et al., 2007). Flavonoids are known to bind strongly to the benzodiazepine site of the GABA receptor (Wasowski and Marder, 2012) hence, these phytochemical compounds as reported in a study that screened the phytochemical constituents of the plant (Al Snafi et al., 2015) may be part of the components of the essential oil eliciting a sedative activity.

CONCLUSION

The study concluded that Asparagus officinalis L. essential oil possesses significant anxiolytic and sedative activities.
REFERENCES

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