In Vitro Anti-Acetylcholinesterase Activity of Dichloromethane Leaf Extracts of Carphalea glaucescens in Chilo partellus Larvae

Njoroge W Anne1*, Ngugi M Piero1, Aliyu Umar1-2 Matheri Felix1, Gitahi S Maina1, Mwangi B Maina1, Njagi J Murithi1, Mworia J Kiambi1 and Ngure G Mutero

1Department of Biochemistry and Biotechnology, Kenyatta University, Nigeria
2Department of Biological Sciences, Bauchi State University Gadau, Nigeria

Abstract

Acetylcholinesterase (AChE) hydrolyses the neurotransmitter acetylcholine resulting in the termination of nerve impulse at the synapse. Anti-acetylcholinesterase activities stop the passage of the nerve impulse at the synapse resulting in continuous stimulation which can lead to death. The manufacturers of many pesticides target the AChE because it interferes with the passage of the nerve impulse. In vitro study revealed that DCM leaf extract of Carphalea glaucescens has anti-acetylcholinesterase activity against crude acetylcholinesterase (AChE) enzyme extracted from Chilo partellus and an IC50 of 12.02 mg/ml was calculated. After qualitative phytochemical screening was carried out the phytochemicals which were present were tannins, phenols, flavonoids, steroids, terpenoids and alkaloids.

Keywords: Chilo partellus; Carphalea glaucescens; Anti-acetylcholinesterase

Abbreviations: AChE: Acetylcholinesterase; ANOVA: Analysis of Variance; DCM: Dicholomethane; WHO: World Health Organization; ACTI: Acetyltiocholine Iodide; DTNB: 5,5'-Dithiobis-(2-Nitrobenzoic Acid)

Introduction

Acetylcholinesterase is the target for insecticides belonging to organophosphorus and carbamate group. Organophosphates act upon the nervous system of the pests interfering with the passage of impulse. Due to the negative effects of synthetic insecticides, there is need to develop cheaper and safer insecticides.

The Chilo partellus (spotted stem borer) is one of the major constraints in maize and sorghum production worldwide. The yield losses reported due to stem borers vary greatly. Melaku [1] reported 49% grain yield losses due to stem borers in northern Ethiopia but on average yield losses can be estimated between 20% and 50%. C. partellus is very invasive and once it invades an area it displaces native species and is widely distributed. In coastal Kenya, there is evidence that C. partellus has partially displaced the indigenous stem borer, Chilo orichalcicilli [2-4].

The distribution of C. partellus now includes Ethiopia, Kenya, Tanzania, Mozambique, Swaziland, Lesotho, and Botswana [5,6]. Different conventional insecticides have been used to control stem borer resulting in high productivity but there are shortcomings in their application, including the high residue levels of pesticides in agricultural produce, pest resistance as well as environmental pollution [7]. The management of C. partellus has been typically carried out by synthetic insecticides, which are non-biodegradable and not environmentally safe [8,9].

In this perspective, plants are considered the alternative sources of insect-pest control because they contain a range of bioactive compounds and many of which are selective. In Africa and elsewhere, plants extracts are still widely used in the treatment of many ailments and up to 80% of the African population use traditional medicines for health care [10]. The present study aimed to investigate the mode of action of C. glaucescens in the inhibition of acetylcholinesterase.
The concentration of the isolated AChE was determined by method described by Bradford [11].

**Determination of AChE concentration**

After crude AChE was extracted from the larvae of *C. Partellus*, the concentration was determined by the method described by Bradford [11] and bovine serum albumen (BSA) was used as the standard for protein. The dye was made by dissolving 100mg coomassie brilliant blue G-250 in 50 ml 95% ethanol followed by 100ml 85% (w/v) phosphoric acid. Absorbance was measured at 595nm and the absorbance values were used to plot the standard curve. The standard curve was used to determine the concentration of the AChE.

**Anti-acetylcholinesterase activity assay**

Acetylcholinesterase activity was determined as described by Ellman method [12], with some modification that allowed the use of 1 ml cuvette glass. In 2 ml eppendorf tube, 150 µl of 14 mM of acetythiocholine iodide was used as substrate to initiate the reaction. The eppendorf tube with the mixture was incubated for 30 minutes at 25°C. The principle was to measure the production of thiocholine from the hydrolysis of acetylthiocholine iodide. Thereafter 10 µl of 10 mM of DTNB was added which was used for the measurement of AChE activity. The reaction mixture was then incubated for 5 minutes at room temperature (25°C). The absorbance is read at 412 nm with spectrophotometer against a blank and reference pesticide used was cyclone. The optical density (OD) was read after one minute and the fourth minutes. Then the change optical density with time (OD/min) was calculated to estimate substrate hydrolysis overtime. One unit of AChE activity is defined as 1 µl of substrate hydrolyzed per minute. The activity of the AChE was calculated using Beer lamberts law.

**Qualitative phytochemical screening**

Qualitative phytochemical screening was done to determine the presence of selected secondary metabolites. This was done according to the standard methods as described by Harborne [13] and Kotake [14].

<table>
<thead>
<tr>
<th>Treatment (mg/ml)</th>
<th>Mean change in absorbance</th>
<th>Enzyme activity (µmoles/min/mg protein)</th>
<th>Mean % inhibition</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal control</td>
<td>0.03±0.058</td>
<td>0.000</td>
<td>0.00±0.00</td>
</tr>
<tr>
<td>Positive control</td>
<td>0.001±0.055</td>
<td>3.744</td>
<td>96.97±1.84</td>
</tr>
<tr>
<td>2.5</td>
<td>0.009±0.003</td>
<td>4.444</td>
<td>73.64±8.67</td>
</tr>
<tr>
<td>5</td>
<td>0.013±0.028</td>
<td>1.836</td>
<td>64.80±28.00</td>
</tr>
<tr>
<td>7.5</td>
<td>0.018±0.002</td>
<td>0.902</td>
<td>45.45±6.19</td>
</tr>
<tr>
<td>25</td>
<td>0.021±0.004</td>
<td>0.021</td>
<td>35.50±13.4</td>
</tr>
<tr>
<td>50</td>
<td>0.028±0.036</td>
<td>0.039</td>
<td>35.09±4.66</td>
</tr>
<tr>
<td>75</td>
<td>0.037±0.033</td>
<td>0.032</td>
<td>34.54±6.05</td>
</tr>
</tbody>
</table>

Values are expressed as mean ± SEM for triplicate readings. Values with different superscripts are significantly different calculated by one way ANOVA followed by Tukey’s post hoc test (p>0.05).

**Table 1**: Effects of DCM leaf extracts of *C. glaucescens* on anti-acetylcholinesterase of *C. partellus* larvae.

![Figure 1: Comparison of percent inhibition at various concentrations of DCM leaf extracts of *C. glaucescens* on anti-acetylcholinesterase of *C. partellus.*](image1.png)

**Figure 1**: Comparison of percent inhibition at various concentrations of DCM leaf extracts of *C. glaucescens* on anti-acetylcholinesterase of *C. partellus.*

![Figure 2: Linear regression graph with line of best fit for calculation IC50.](image2.png)

**Figure 2**: Linear regression graph with line of best fit for calculation IC50.

**Data collection, management and statistical analysis**

Data collected included the efficacy of the extract against the AChE, change in absorbance, and qualitative phytochemical screening was also done. Data on phytochemical was presented on a table showing presence or absence of the phytochemicals. Data on different extract concentrations, and controls were analyzed using one way ANOVA, which was followed by Tukey’s post hoc for pairwise separation and comparison of means. Minitab version 17 software was used for statistical analysis.

**Results and Discussion**

The DCM leaf extract of *C. glaucescens* showed efficacy against AChE activity in *C. partellus* as indicated by percent inhibition of the enzyme activity by various extract concentrations (Table 1 and Figure 1) using method described by [12]. Acetylcholinesterase plays a critical role in terminating synaptic transmission so that the next nerve impulse can be transmitted across the synapse. Therefore, the leaf extract has the potential to prevent the AChE of *C. partellus* in termination of nerve impulse. Among the six different extract concentrations, the 2.5 mg/ml had the highest percent inhibitory effects on activity of acetylcholinesterase with a value of 73.64% and 75mg/ml had the lowest percentage enzyme activity inhibition of 34.54% (Table 1 and Figure 2). The normal control group had no effect against the enzyme activity. This is similar to a study conducted by Orban [15], which demonstrated that all *Fumaris* species studied showed the most potent inhibitory activity against AChE. The high SEM for concentration 5mg/ml (Figure 1) can be interpreted to mean that it was not a representative of the population however despite the high SEM the results were consistent with results of other concentrations.

From this study, any change in absorbance was also associated
with inhibition of enzyme activity. The IC\textsubscript{50} for DCM leaf extract of C. glaucescens was computed by regression line and found to be 12.02 mg/ml (Figure 2). At lower extract concentrations the extract was more sensitive to enzyme activity than at higher concentrations (Figure 3) which mean that to be effective only low concentrations of the extract would be required.

Results revealed that anti acetylcholinesterase activity of DCM leaf extracts of C. glaucescens in C. partellus is dose dependent because the extract concentration increased the percent enzyme inhibition decreased. This was comparable to a study by [16] which observed that Foliar application of semi-solid crude extract of T. orientalis on maize was found to be effective against C. partellus. In this study, the leaf extract showed enzyme inhibitory effects. The inactivated enzyme is no longer capable of hydrolyzing acetylcholine, resulting in the build up of ACh in the nerve synapse, leading to death [17,18].

Qualitative phytochemical screening

In DCM extract of C. glaucescens phytochemicals which were present after screening were tannins, phenols, flavonoids, steroids, terpenoids and alkaloids. However saponins and cardiac glycosides were absent (Table 2). The bioactivity of DCM leaf extracts of C. glaucescens can be attributed to constituent phytochemicals such as phenols and flavonoids in the extracts which are associated with phenols and flavonoids [19]. Similarly, a study conducted by [15] found out that since most of the acetylcholinesterase inhibitors are known to contain nitrogen, the higher activity of these extracts may be due to their rich alkaloidal content.

Conclusion

In conclusion, this study has revealed that the DCM leaf extracts of C. glaucescens has the potential of in vitro anti acetylcholinesterase activity in C. partellus and it is possible for the studied plants to possess bioactive compounds. Therefore C. glaucescens can be used as biocide in the control of C. partellus. However the bioactivity could have been higher if pure extract were used and would recommend purification of AChE through processes such as chromatography.

Acknowledgement

Thanks are due to Mr. Daniel Gitonga for providing technical support.

References