Investigation of the Cyclooxygenase Inhibition by Polyphenol Extracts derived from Monocultivar oils from Olive Tree Varieties from the Maltese Islands

Gatt Lucienne1, Schembri-Wismayer Pierre2 and Zammit-Mangion Marion3

1,3 Department of Physiology and Biochemistry, Faculty of Medicine and Surgery, University of Malta, Msida, MSD 2080
2 Department of Anatomy, Faculty of Medicine and Surgery University of Malta, Msida, MSD 2080

Contacting author: lgat0008@um.edu.mt, 0035623402799

INTRODUCTION

Cyclooxygenase (COX) is the enzyme responsible for the conversion of twenty carbon fatty acids such as arachidonic acid (AA) into prostaglandins (PGs). In humans, PGs play a role in various important processes such as inflammation, labour initiation, kidney function and blood clotting (Dubois et al., 1998). Today it is recognised that inflammation plays an important role in leading to numerous other diseases, such as cancer (Rakoff-Nahoum, 2007). Hence there is interest in modulating this pathway through natural means. NSAIDs cause renal and gastrointestinal side effects, such as damage to the mucosa and ulceration (Hawkey, 2001). In this study, the inhibition of COX by polyphenols extracted from autochthonous Maltese olive oil varieties was investigated.

METHODOLOGY

1. Extraction of polyphenols

Polyphenols were extracted from moncultivar olive oil through liquid-liquid separation using a 60:40 methanol:water mixture. The aqueous layer was collected and methanol was evaporated using a rotary evaporator set at a temperature of 40 °C (Papadopoulou and Boskou, 1991). The sample was freeze-dried, and then dried under nitrogen.

2. Extract analysis

The total phenol content of the extracts was determined using the Folin-Ciocalteau test as reported by Slinkard and Singleton (1977), the only change being a reduction in the volumes as reported by Waterhouse (2001). Different concentrations of gallic acid were used as standards. The absorbance of the standards and samples was read at 765 nm. The phenol content of the dried extracts was calculated using the gallic acid standard curves. The experiment was repeated three times to ensure result repeatability.

3. COX inhibition

The percentage inhibition of the extracts on cyclooxygenase was determined using the Cayman COX inhibitor screening assay kit. PG standards supplied with the kit were used to prepare the standard curve required. Acetylsalicylic acid was used throughout, as a positive control. The final concentration of both the extracts and acetylsalicylic acid was 0.2 ppm.

RESULTS

Table 1: The % inhibition of COX 1 and 2 using two polyphenol extracts. Acetylsalicylic acid represents the positive control for COX inhibition. The results are reported for both the control and the extracts at final concentrations of 0.2 ppm.

<table>
<thead>
<tr>
<th>Sample</th>
<th>Enzyme</th>
<th>% Inhibition</th>
</tr>
</thead>
<tbody>
<tr>
<td>Extract 2</td>
<td>COX 1</td>
<td>45.03</td>
</tr>
<tr>
<td>Extract 2</td>
<td>COX 2</td>
<td>35.34</td>
</tr>
</tbody>
</table>

Table 2: A graph of % B/B0 against log PG concentration of standards (μg/ml) representing an average standard curve for the two sets of standards. Error bars represent standard error.

![Figure 3](image-url)

The COX inhibition results are shown in Figure 3 and Table 2. Table 2 shows that Extract 1 (Maltese native olive oil variety) provides higher inhibition of COX 1 and COX 2 when compared to Extract 2 (Italian olive oil variety). In fact, the effect of Extract 1 on COX 1 is 55% that of the positive control while for COX 2, it is 49% that of the control. Moreover, while Extract 1 inhibits COX 1 and COX 2 to a lesser degree than the positive control, it is relevant to highlight that while acetylsalicylic acid is composed of just the active component, Extract 1 is a crude polyphenol extract with no purification.

CONCLUSIONS

From this investigation, one can conclude that Extract 1 is higher in phenol content than Extract 2. Moreover, at a final concentration of 0.2 ppm, Extract 1 appears to be a better inhibitor of COX 1 and COX 2 than Extract 2. Purification of the extract to determine the active component giving rise to this inhibitory effect will follow.

ACKNOWLEDGEMENTS

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REFERENCES


