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

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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. Two isoforms of the enzyme exist, these being COX-1 and COX-2. While the two are similar in primary protein structure and in the catalytic reaction they perform, they differ in their tissue expression patterns, inhibitor selectivity and in the substrates they utilise. Moreover, while COX-1 is constitutive, COX-2 is inducible (Vane *et al*., 1998). Non steroidal anti-inflammatory drugs (NSAIDs) inhibit COX, resulting in a decline in PG formation. Though greatly beneficial, 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 autochtonous Maltese olive oil varieties was investigated.

METHODOLOGY

1. Extraction of polyphenols

Polyphenols were extracted from monocultivar 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 (Papadopoulos and Boskou, 1991). The sample was freeze dried, and then dried under nitrogen.

3. 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 repeatibility.

5. 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.

Figure 1: A flowchart of the methodology used in this study

RESULTS

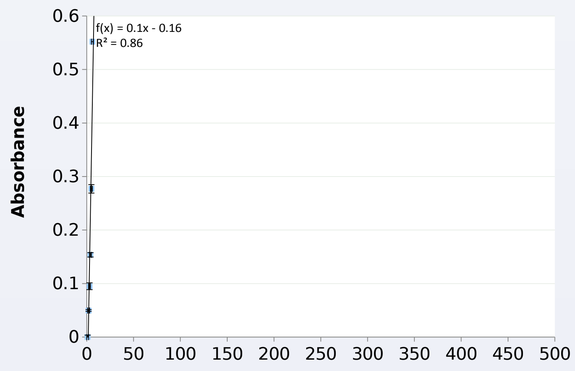

Figure 2: A graph of absorbance against concentration of Gallic acid (mg/L) representing an average standard curve for the three sets of gallic acid standards. Error bars represent standard error.

Extract	Mean phenol content (n=3)	Mean original phenol content(Gallic acid equivalents, mg/L)
Extract 1	388.18	3881.82
Extract 2	282.12	2821.21

Table 1: The phenol content of each extract as determined by the Folin-Ciocalteau test, expressed as gallic acid equivalents in mg/L.

The results of the **Folin-Ciocalteau test** are shown in **Figure 2 and Table 1**. This colorimetric assay works on the premise that light absorbance increases as the amount of hydroxyl groups in a sample increase. Table 1 shows the average phenol content of the two polyphenol extracts used in this study. The final column in this table accounts for the 1 in 10 dilution required for the concentration of the extracts to fall within the range of the three gallic acid standard curves (Figure 2). From the results obtained, one can conclude that, following extraction, Extract 1 (Maltese native olive oil variety) had the highest phenol content, followed by Extract 2 (Italian olive oil variety).

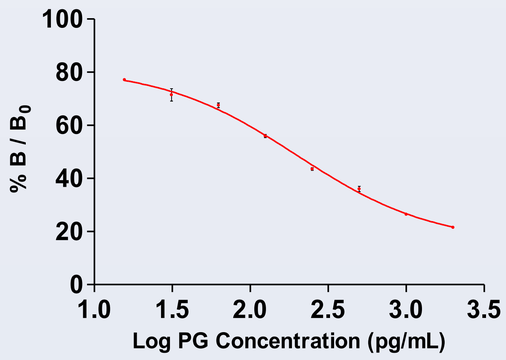

Figure 3: A graph of % B/Bo against log PG concentration of standards (pg/L) representing an average standard curve for the two sets of standards. Error bars represent standard error.

Sample	Enzyme	%Inhibition
Acetylsalicylic acid	COX 1	82.61
	COX 2	72.17
Extract 1	COX 1	45.03
	COX 2	35.34
Extract 2	COX 1	22.76
	COX 2	29.3

Table 2: 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.

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. Contrastingly, the effect of Extract 2 on COX 1 is 28% that of the positive control and for COX 2, it is 41% that of the positive 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.

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