REPORT

Evaluation of anti-inflammatory activity of selected legumes from Pakistan: *In vitro* inhibition of Cyclooxygenase-2

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Abstract: Crude methanolic extracts of selected legumes namely, black gram (*Vigna mungo* L.), green gram (*Vigna radiata* (L.) R. Wilczek), soybean (*Glycine max* (L.) Merr.) and lentil (*Lens culinaris* Medik.) were investigated for anti-inflammatory effects, using COX-2 producing PGE_2 inhibitory assay. Percentage inhibition observed was 73.93, 79.84, 92.17 and 74.47 for black gram, green gram, soybean and lentil respectively at 20µg/ml extract concentration. The 100µg/ml concentration showed increase in the percent inhibition except for soybean. This is the first report on COX-2 inhibitory potential of food legumes.

Keywords: Anti-inflammatory, legumes, COX-2, lentil, soybean, black gram, green gram.

INTRODUCTION

Inflammation is basic defense mechanism that plays a key role in protection from infection, burn, toxic chemicals, allergens or other noxious stimuli. However, it should not be persistent and should be in limits otherwise may lead to many chronic illnesses (Kumar *et al.*, 2004). All inflammation-causing stimuli, activate cell membrane (enzyme) phospholipases that convert phospholipids into arachidonic acid (AA). AA act as substrate for cyclooxygenase or prostaglandin H synthetase, leading to productions of various types of prostaglandins (PG_2, PGE_2, $PGF_{2\alpha}$, $PGI_{2)}$ and thromboxane A_2 which are important mediators of inflammation (Górski *et al.*, 2001). Medical markets have plenty of synthetic anti-inflammatory medicines which are not without side effects. So world is now turning towards natural anti-inflammatory agents derived from plants as these are comparatively safe, cost effective and locally available.

Leguminosae is an important plant family, various species of which are grown in different parts of Pakistan in abundance. Food legumes commonly known as pulses are used as food commodity as these provide a range of essential nutrients like protein, low glyceamic carbohydrates, dietary fibre, minerals and vitamins making them one of the best sources of all these constituents for poor people. Epidemiological studies indicate that consumption of legumes can play a role in preventing chronic disease, including cardiovascular disease, diabetes and overweight, as well as improving gut health (Kushi *et al.*, 1999). Although some work exists on nutritional and compositional studies of legumes (Zia-Ul-Haq *et al.*, 2007, 2008 a, b; 2009; 2010; 2011a, b, c), however their biological screening has been totally neglected. So in current study we have investigated COX inhibition activities of methanolic extracts of some of these legumes.

MATERIALS AND METHODS

Black gram (*Vigna mungo* L.) (BG), green gram (*Vigna radiata* (L.) R.Wilczek) (GG), soybean (*Glycine max* (L.) Merr.) (S) and lentil (*Lens culinaris* Medik.) were collected, chopped and soaked in methanol for about fifteen days, filtered through Whatman filter paper, and concentrated using Rotavapor. The obtained semi-liquid was further dried in dessicator and the extract obtained was used in COX analysis.

The extracts were tested according to reported procedure (Reininger and Bauer, 2006) using human recombinant COX-2 enzyme. COX-2 (0.5 unit/ reaction) was added to 180 µl of incubation mixture consisting of tris buffer (pH 8.0; 100 mM), porcine hematin (5 µM), L-epinephrine (18 mM) and Disodium EDTA (50 µM). All extracts to be tested were dissolved in DMSO and 10 µl was added to the mixture pre-incubated for 5 min at room temperature.

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The reaction was initiated by the addition of arachidonic acid (5 µl; 10 µM) and stopped by the addition 10 µl of 10% formic acid after 20 min incubation at 37ºC. DMSO 10 µl was used as blank. All extracts were diluted at ratio of 1:15 in assay buffer; the concentration of PGE_2 produced was determined using PGE_2 EIA kit following manufacturer instructions. Microplate reader at wavelength 405 nm was used to measure absorbance that is relative to PGE_2 concentration. The percentage inhibitions of PGE_2 formation were calculated from treated and untreated samples (blanks). Two independent experiments with two replicates were performed.

RESULTS

In present work we evaluated the methanolic extract of four legumes in COX-2 inhibitory assay; we used 20µg/ml of the extracts, we found in our study that BG had 73.93%, GG 79.84%, S 92.17% and L 74.47% of inhibition while 84.45%, 82.867%, 85.11% and 94.54% at 100µg/ml (Fig. 1). Indomethacin (IC_{50} value 1.22µM) was used as non-selective COX inhibitor in the assay. Soybean extract had the highest inhibitory activity while black gram had the least.

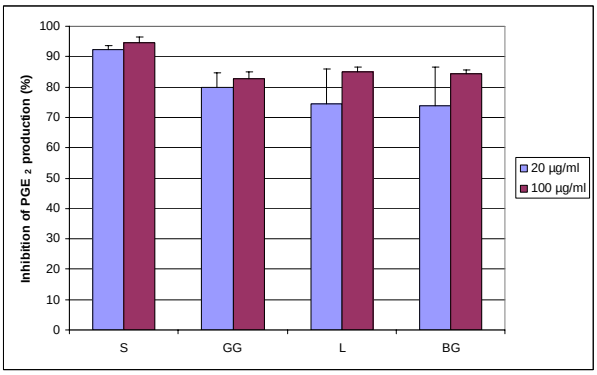

Fig. 1: Inhibitory activity of four methanolic extracts at concentrations 20 and 100 µg/ml on PGE_2 production catalyzed by COX-2. S: soybean; GG: green gram; L: lentil; BG: black gram

DISCUSSION

Cyclooxygenases (COX) are responsible for the production of prostaglandins (PGs). COX are of two types; COX-1 that is expressed in many cells and its activity is concerned with important normal physiological processes while COX-2 is selectively expressed in inflammatory site or tissue. So in modern approach of anti-inflammatory therapy, COX-2 is selectively blocked to avoid untoward effect as result of COX-1 blockage (Seibert and Masferrer, 1994).

It was reported previously that legumes have preventive role in breast, prostate and stomach cancer (Qin *et al.,* 2006; Trock *et al.,* 2006; Yan and Spitznagel, 2009) which may be probably due to the presence of various antioxidants (Faris *et al.,* 2009). Various oncology researches also suggest COX-2 involvement in the breast cancer, with the increase level of PGs (Arun and Goss, 2004). So targeting COX-2 pathway in cancer therapy had promising results in clinical trials (Méric *et al.,* 2006). In conclusion, the extracts from four legumes (soybean, black gram, lentil, and green gram), showed promising COX-2 inhibitory activity *in vitro*. These results indicate possible positive effects of legumes in prevention of diseases connected with COX-2 activity. However, further research should identifies the compounds responsible for their activity and confirm the activity in other types of assay.

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