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PLATELET AGGREGATION AND ANTI-INFLAMMATORY EFFECTS OF GARDEN PEA, DESI CHICKPEA AND KABULI CHICKPEA

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Abstract: Inflammation is the natural body defense mechanism for the removal of injurious agents, necrosed cells and tissues from the body. This study was aimed to evaluate the anti-inflammatory and platelet aggregation effects of three medicinal plants of Pakistan. Methanolic extract of Garden pea inhibited arachidonic acid (AA)-induced platelet aggregation (IC_{50} = 35 µg/mL) and platelet activating factor (PAF)-induced platelet aggregation (IC_{50} = 38 µg/mL) in a dose dependent fashion. Methanolic extract of Desi chickpea inhibited arachidonic acid (AA) induced platelet aggregation (IC_{50} value = AA = 46 µg/mL) in dose dependent fashion while was found not active against PAF-induced platelet aggregation. Methanolic extract of Kabuli chickpea was found not active against both arachidonic acid (AA)-induced platelet aggregation and PAF-induced platelet aggregation. The best potential to inhibit *in vitro* COX-2 activity showed garden pea (*Pisum sativum*; the synthesis of PGE_2 reduced by 92% in comparison with untreated control wells) followed by Desi chickpea (*Cicer arietinum* var; 87% inhibition) and Kabuli chickpea extracts (*Cicer arietinum* var; 65% inhibition). All extracts were tested at concentration 20 µg/mL. in COX-2 assay.The results indicate that if the same were happening *in vivo*, Garden pea, Desi chickpea and Kabuli chickpea could be useful as natural antithrombotic anti-inflammatory materials.

Keywords: platelet aggregation, Garden pea, Desi chickpea, Kabuli chickpea, cardiovascular disease

Inflammation is a non-specific, defensive response of the body to tissue damage. The inflammatory response mobilizes the body's defenses, isolates and destroys microorganisms and other injurious agents. Anti-inflammatory activity of an extract can be determined by their ability to inhibit PGE_2 production catalyzed by COX2 (1). Natural antithrombotic agents that influence platelet function are of potential interest for primary prevention of cardiovascular disease (CVD) (2). In the United States, Chickpeas (*Cicer arietinum L.*) are known as garbanzo beans and are an important source of protein in human diets. It is believed that chickpeas originated in Turkey. They have been cultivated in India, Middle East, the Mediterranean and Ethiopia since ancient times and were brought to the New World through trade and subjugations. Chickpea seed contains 13 to 33% protein, 40 to 55% carbohydrates, 4 to 10% oil and fatty acids (50% oleic and 40% linoleic) (3). In addition to nutritional impor-

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tance, Garden pea is also important due to distinctive medicinal properties. Its seeds are sweet, farinaceous and edible. Pulse is nutritious, refrigerant, appetizer, and used against skin burning. Its soup is light to digest, refrigerant, astringent and beneficial in diseases of blood, phelgem and vitiated bile. The seeds are also noted to possess good antimicrobial properties (4) Pulse is believed to cause dysentery when eaten raw (5). Oil from ripened seed has anti-sex hormonic effects; produces sterility and antagonizes effect of male hormones (6). Seeds are thought to cause dysentery when eaten raw. In Spain, flour is considered emollient and resolvent, applied as a cataplasm. It has been reported that seeds contain trypsin and chymotrypsin, which could be used as contraceptive, ecbolic, fungistatic and spermicide (7). The dried and powdered seed has been used as a poultice on the skin, where it has an appreciable affect on many types of skin deseases including acne (8). The oil from the seed, given once a month to women, has shown promise of preventing pregnancy by interfering with the working of progesterone (6). The oil inhibits endometrial development (9). Its wood is used for membrane stabilizing action, and has carminative, diuretic, immunomodulatory and diaphoretic activity (10). The raw peas (*Pisum sativum* L.) have been found to reduce plasma total and LDL cholesterol and hepatic esterified choles-

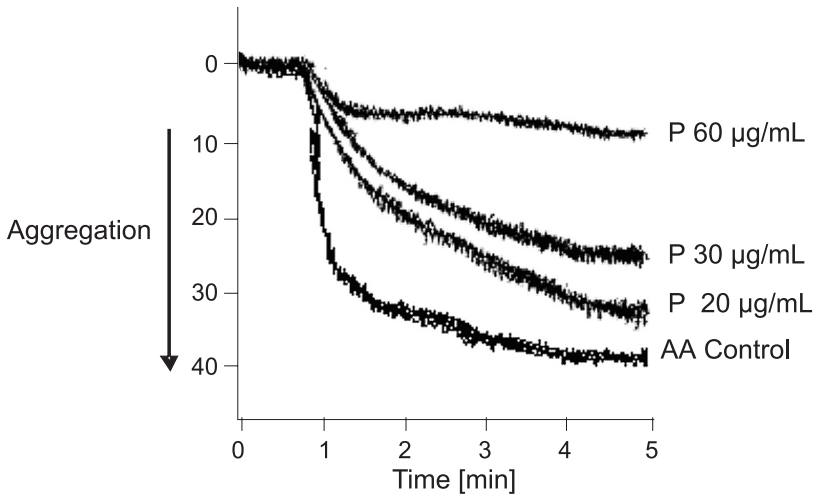

Figure 1. Tracings of inhibition of the platelet aggregation by Garden pea, against AA. Control is the aggregation curve obtained by adding AA (1.8 mM) and was taken as 100% aggregation. (IC_{50} value = AA = 35 µg/mL)

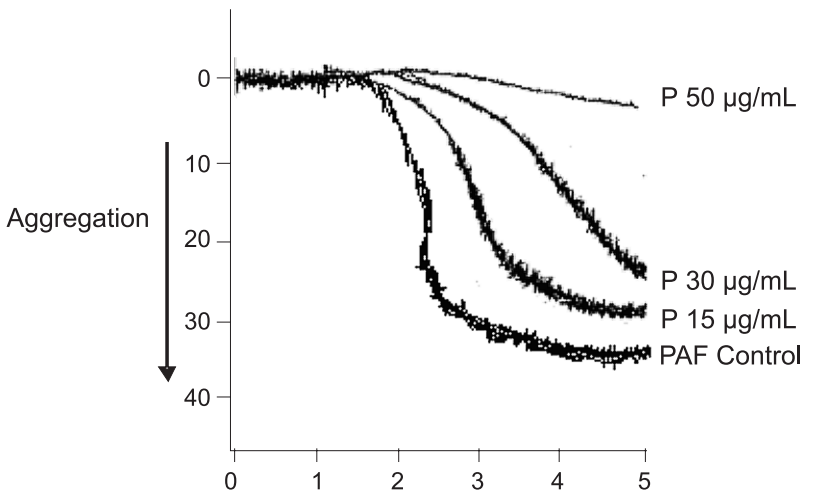

Figure 2. Tracings of inhibition of the platelet aggregation by Garden pea, against PAF . Control is the aggregation curve obtained by PAF (0.8 µM) and was taken as 100% aggregation. (IC_{50} value = PAF = 38 µg/mL)

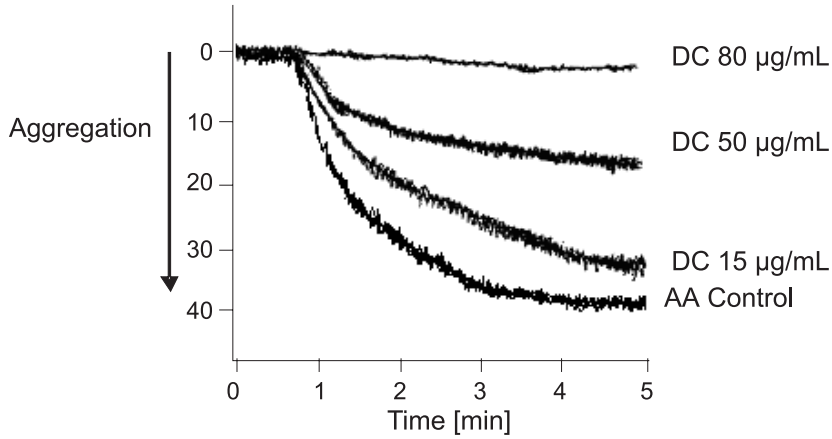

Figure 3. Tracings of inhibition of the platelet aggregation by Desi Chickpea against AA. Control is the aggregation curve obtained by adding AA (1.8 mM) and was taken as 100% aggregation. (IC_{50} value = AA = 46 µg/mL)

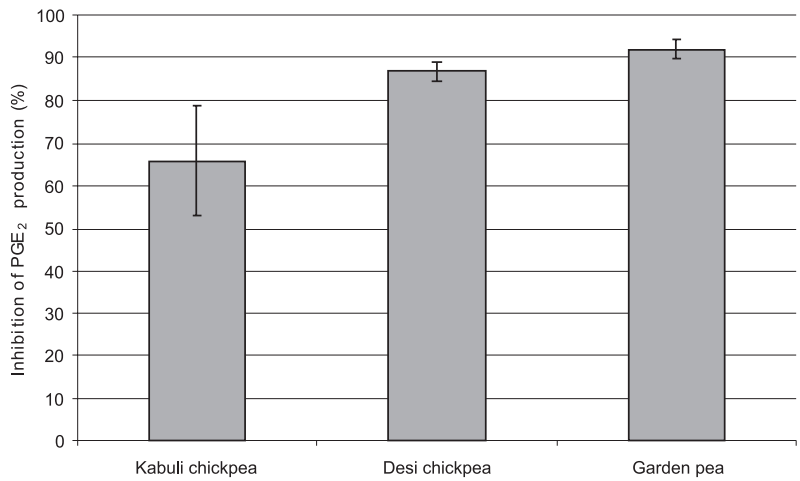

Figure 4. Extracts tested at concentration of 20 µg/mL for inhibition of *in vitro* PGE_2 production catalyzed by COX-2

terol in intact and ileorectal anastomosed pigs fed cholesterol-rich diet (11).

As part of our studies to explore the flora of Pakistan (12–18) we have explored anti-inflammatory and platelet aggregation activities of common legumes of Pakistan.

MATERIALS AND METHODS

Plant material

Seeds of Garden pea, Desi chickpea and Kabuli chickpea were obtained from department of agronomy Bahauddin Zikarya University, Multan. Seeds of all three plants were stored in stainless-steel containers at 4°C prior to use.

Extraction

The seeds were chopped, ground to coarse powder and macerated with methanol for 2 days at room temperature. The methanolic extract was evaporated under reduced pressure leaving behind a brown semisolid mass.

Platelet aggregation

Hussain et al. described platelet aggregation which is illustrated here briefly. Blood was taken

from undiseased human volunteers who have not taken any medication two weeks prior to participation in the study. The blood was mixed with 3.8% (w/v) sodium citrate solution in a ratio of 9:1 and centrifuged at 260 × g for 15 min at 20°C in order to obtain platelet rich plasma. The remaining blood was centrifuged at 1200 × g for 10 min to obtain platelet poor plasma (PPP). Phase contrast microscopy was carried out to determine platelet count. Aggregation was carried out at 37°C with platelet rich plasma having platelet counts between 2.5 and 3.0 × 108/mL of plasma. Dual-channel Lumi aggregometer-400 (Chronolog Corporation, Chicago, USA) was used to monitor aggregation. Aggregation was induced with arachidonate (1.8 mM) or platelet activating factor (0.8 µM). The anti-aggregatory effect of methanolic extract of Garden pea, Desi chickpea and Kabuli chickpea were studied by addition of platelet activating factor and arachidonate (aggregation agents). The resulting aggregation was recorded for 5 min after challenge, by the change in light transmission as a function of time (19).

Evaluation of anti-inflammatory activity

Owing to adverse reactions of synthetic and chemical medicines, herbal medicines have drawn an attention to be used to improve our basic health needs. Several botanicals and herbs such as olive oil, clove oil, ginger and turmeric have been shown to exhibit effective anti-inflammatory effect. In this study, anti-inflammatory activity was carried out using COX-2 inhibition method. The extracts were tested according to procedure described previously by Reininger and Bauer (20) with human recombinant COX-2 (Sigma-Aldrich, MO, USA). COX-2 (0.5 unit/reaction) was added to 180 µL of incubation mixture consisting of 100 mM Tris buffer (pH 8.0), 5 µM porcine hematin, 18 mM L-epinephrine and 50 µM Na_2EDTA (all Sigma-Aldrich). Tested extracts dissolved in DMSO or pure DMSO (in case of blank) were added (10 µL) to the mixture and preincubated 5 min at room temperature. The addition of 5 µL of 10 µM arachidonic acid started the reaction. After 20 min incubation at 37°C, the reaction was stopped by 10 µL of 10% formic acid. All samples were diluted 1 : 15 in assay buffer and concentration of PGE_2 produced by the reaction was determined by PGE_2 EIA kit (Assay Designs, MI, USA) according to the manufacturer instructions. Absorbance relative to PGE_2 concentration was measured by microplate reader Tecan Infinite M200 (Tecan Group, Switzerland) at 405 nm. The results were expressed as a percentage inhibition of PGE_2 formation against untreated samples (blanks). Two independent experiments with two replicates were performed (20).

RESULTS AND DISCUSSION

Platelet aggregation

Methanolic extract of all peas were scrutinized for human platelet aggregation effects. Platelet aggregation was induced by arachidonic acid (AA) and platelet activating factor (PAF). Methanolic extract of Garden pea inhibited arachidonic acid (AA) induced platelet aggregation in a dose dependant fashion i.e., the effect increased for AA from 20, 30 and 60 µg/mL. Concentration of methanol extract of Garden pea required to inhibit AA induced human platelet aggregation by 100% (IC_{50}) was found to be AA = 35 µg/mL. Similarly, methanolic extract of Garden pea also showed dose dependent platelet aggregation effects i.e., the effect increased for PAF from 15, 30 and 50 µg/mL PAF-induced platelet aggregation in a dose related manner with IC_{50} of 38 µg/mL (Figs. 1 and 2). Arachidonic acid (AA) induced platelet aggregation effects for methanolic extract of Desi chickpea was also shown to be dose dependent i.e., the effect increased for AA from 15, 50 and 80 µg/mL. Concentration of methanol extract of Desi chickpea required to inhibit AA induced human platelet aggregation by 100% (IC_{50}) was found to be AA = 46 µg/mL (Fig. 3). It was observed that Desi chickpea was not active against platelet activating factor (PAF)-induced platelet aggregation. In this study, it was found that methanolic extract of Kabuli chickpea did not show any effects against both arachidonic acid (AA)-induced and platelet activating factor (PAF)-induced platelet aggregation.

The mechanism behind these effects is yet not clear but it might be said that the compound(s) responsible for these effects are methanol-soluble, heat-resistant plant botanicals, which might be different from chemical anti-coagulating agents (salicylates), and might also be due to yet undisclosed compounds similar to nicotinic acid and known xanthines. One postulated mechanism may be that the peas extract decreased the conversion of [^{14}C]-arachidonic acid to thromboxane B2 by the platelets. These studies show that peas extracts contain compounds, which are energetic in restraining platelet aggregation (21).

Anti-inflammatory activity

The methanolic extracts of all three peas were tested *in vitro* for inhibition of PGE_2 synthesis cat-

alyzed by COX-2 using purified human recombinant enzyme. Indomethacin (standard non-selective COX inhibitor) was used as reference inhibitor (IC_{50} = 1.22 µM). Extracts from Garden pea and Desi chickpea tested at concentration of 20 µg/mL reduced PGE_2 production by more than 85% in comparison with the reactions without inhibitor (blanks). Kabuli chickpea showed moderate reduction of activity (about 65%; Fig. 4). Further research should reveal if the inhibitory activity of tested extracts is caused by possible presence of fatty acids in the extracts, which are known *in vitro* COX-2 inhibitors (e.g., linoleic acid) (20) or if the activity resulted from presence of other compounds contained in peas.

CONCLUSION

The program initiated at the end of 20th century to discover new cardiovascular and other pharmacological active botanicals is continued today. Many novel lead compounds from plant botanicals have been arised from this program. The purpose of this research was also to provide some preliminary study of pea plants for platelet aggregation and anti-inflammatory activity and based on these results, we can say that the Garden pea, Desi chickpea and Kabuli chickpea show promising platelet aggregation and anti-inflammatory activity and if the same would be happening *in vivo*, Garden pea, Desi chickpea and Kabuli chickpea could be useful as natural antithrombotic anti-inflammatory materials. As a footnote we would like to say that this suppression of platelet function could be beneficial in preventing thrombotic and proinflammatory events associated with activated platelets. Preliminary results recorded in enzymatic *in vitro* COX-2 assay indicate potential of pea extracts to inhibit activity of COX-2. Further studies will be needed to elucidate which compounds are responsible for inhibitory activity of the extracts.

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