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Inhibitor(S) of prostaglandin endoperoxide synthase and lipoxygenase activity in extracts of barley

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INHIBITOR(S) OF PROSTAGLANDIN ENDOPEROXIDE SYNTHASE AND LIPDXYGENASE ACTIVITY IN EXTRACTS OF BARLEY

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ABSTRACT

Aqueous extract of barley was found to inhibit PG endoperoxide synthase and lipoxygenase enzymes responsible for arachidonic acid metabolism. Only 6% (v/v) of aqueous extract produced 50% inhibition of PG endoperoxide synthase while five times more extract was requited to produce 50% inhibition of lipoxygenase. Boiling barley extract did not destroy the ability of the extract to inhibit the enzymes. Fractionation of barley with organic solvents, petroleum ether, chlorform, ethylactate and acetone exhibited selectivity in its action in that foamy layer formed during petroleum ether and ethyl acetate extraction inhibited PG endoperoxide synthase completely whereas lipoxygenase activity was only partially inhibited. These results suggest the *presence* of inhibitor(s) of arachidonic acid metabolism in extracts of barley.

Introduction

Recently a number of plant products have been under investigation in an effort to determine their medicinal value. Inhibitory effects of garlic, onion and ginger on platelet aggregation and arachidonic acid (AA) metabolism in animals and humans have been reported (Bordia, 1978; Srivastava, 1984; Srivastava, 1984a). Also, a colloidal fraction of oats, branded as AVEENO by Knox Laboratories Limited, U.K., has been claimed to relieve inflammatory conditions of the skin. In addition, extract of oats has also been shown to inhibit PG biosynthesis (Saeed et al; 1981).

Barley, a plant product obtained from Hordium Vulgate, is widely used as constituent of cereals (Encyclopedia Britannica, 1980; Encyclopedia Americana, 1985). Its medicinal value in urethral inflanunation, jaundice and indigestion is known among Asians, though not experimentally established yet. Since effect of barley on AA metabolism has not been studied, we investigated effects of aqueous barley extract and its various fractions on AA metabolising enzymes, PG endoperoxide synthase and lipoxygenase. Platelets were taken as a model to measure the activity of PG endoperoxide synthase since this enzyme is present in platelets and leads to the conversion of arachidonic acid to PG endoperoxides and thromboxanes; the proaggregatory substances (Harnberg et al, 1975; Hammerstrom et al 1980). Lipoxygenase was obtained commercially to study the effects of barley.

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Materials and Methods

Barely seeds were purchased from a local market. Sodium salt of arachidonic acid (Type I, 99% pure) and soyabean lipoxygenase were obtained from Sigma, St. Louis, U.S A. Ethanol, chloroform petroleum ether, ethylacetate, acetone, sodium phosphate (monobasic & dibasic) and sodium carbonate were purchased from E. Merck, Germany.

Solution of sodium arachidonate was prepared by dissolving a 10 mg ampoule in 20 µl of ethanol and diluted with 730 µl of 0.2%; w/v sodium carbonate in nitrogen atmosphere.

i) Preparation of aqueous *extract of barley:*

500 grains of barley seeds were soaked in 1 liter of distilled water for 3 days. After 3 days the mixture was shaken vigorously, allowed to settle down. Supernatant was decanted and evaporated under high pressure rotary evaporator at 55^{o}C. The material left behind was thick, brown fluid, that was treated as an extract.

ii) Fractionation of barley:

Step I: 1.5 kg of barley seeds were minced in 5 liters of distilled water and filtered through a fine sieve. Filtrate was shaken vigorously and 100 ml was reserved as fraction 1. Two liters of the filtrate was stored in a refrigerator at 4°C for 18 hours and rest was extracted as described in Step II. After 18 hours, the portion kept in the refrigerator was decanted slowly to get a clear water fraction leaving insoluble material at the bottom. The decanted layer was filtered through a filter paper and 100 ml was numbered fraction 2. Rest of the material was reserved for acetone precipitation. The residue was mixed with 2 litres of distilled water, boiled for 10 minutes and allowed to settle. Supernatant was decanted, concentrated by evaporation and kept as fraction 3. The starchy left over was stored and later labelled as fraction 14.

Step II: Reserved material from Step I was subjected to organic extraction. It was mixed with an equal amount of petroleum ether, shaken vigorously and separated into three layers i.e. etherial, aqueous and foamy. Etherial layer was evaporated to dryness, powder redissolved in distilled water and stored as fraction 4. Aqueous layer was subjected to extraction again with chloroform. Chloroform layer was separated, evaporated to dryness, remaining material redissolved in distilled water and was stored as fraction 5. The foamy layer was also evaporated to dryness and residue redissolved in distilled water and was stored as fraction 12. The aqueous layer was again extracted with ethyl acetate and the three layers were separated. Ethyl acetate was evaporated to dryness, residue redissolved in distilled water and stored as fraction 6. The foamy layer was evaporated to remove organic traces, redissolved in distilled water and stored as fraction 13. An aliquot of 100 ml of aqueous layer was concentrated by evaporation and stored as fraction 8.

Step III: 300 ml of the remaining aqueous from Step II was mixed with 600 nil of acetone, filtered evaporated to dryness residue redissoved in distilled water and stored as fraction 7. The precipitate was dissolved in distilled water and stored as fraction 9.

200 ml aliquot of fraction 2 was also precipitated with acetone and filtered. Filtrate was concentrated by evaporation and stored as fraction 10. Residue was dissolved in distilled water and stored as fraction 11.

Inhibition of PG endoperoxide synthase:

Platelet rich plasma (PRP) was prepared from human blood drawn from healthy volunteers who denied taking any medicine within a week of venepuncture. Blood, to which sodium citrate was added, was centrifuged at 250 xg for 20 minutes at room temperature. An aliquot of PRP was retained for testing and the remaining was centrifuged at 1000 xg for 10 minutes to obtain platelet poor plasma (PPP). Platelet counts were performed by phase contrast microscopy.

To study the effect of barley extract (BE) on PG endoperoxide synthase, platelet aggregation was measured in a dual channel Lumi-aggregometer (Model 400, Chronolog, USA), using a 0.45 ml PRY. PRP was incubated with BE for 1 minute at 37°C and then challenged with sodium arachidonate. The resulting aggregation was recorded for 4 minutes using a chart recorder and quantified as percent inhibition compared with controls.

Inhibition of lipoxygenase:

Oxygen consumption was used as an index of lipoxygenase activity. Effect of barley was measured polarographically using Biological oxygen monitor (Model 53), Yellow Springs Instrument Co., Ohio USA). A standard assay procedure was developed by selecting optimal conditions for oxygen consumption during the oxidation of AA by lipoxygenase. BE was incubated for 2 minutes at 37°C in a mixture containing 50 mM phosphate buffer (pH 7.4) and 61 pM sodium arachidonate. Assay volume was adjusted to 4 ml, A change is saturation of oxygen, by mixture, was recorded after 8 minutes. Controls contained all the reagents except barley extract.

Results and Discussion

Aqueous extract of barley, as outlined in (i) in Methods section, in concentrations of 5, 8, and 10% (v/v) inhibited PG endoperoxide synthase (assessed by inhibition of platelet aggregation) by 7, 52 and 91% respectively (Fig. 1). Same extract in concentration of 10, 20, 30 and 40% (v/v) inhibited lipoxygenase (assessed by oxygen consumption) by 12, 30, 51 and 72% respectively. The extract of barley was significantly more potent ($P < 0.01$) in inhibiting PG endoperoxide synthase (IC 50 = 6% v/v) than inhibiting lipoxygenase (IC 50 = 29% v/v). It is interesting that after boiling barley

extract retained this inhibitory effect with almost the same potency for both the enzymes (Fig. 2, fraction No.3).

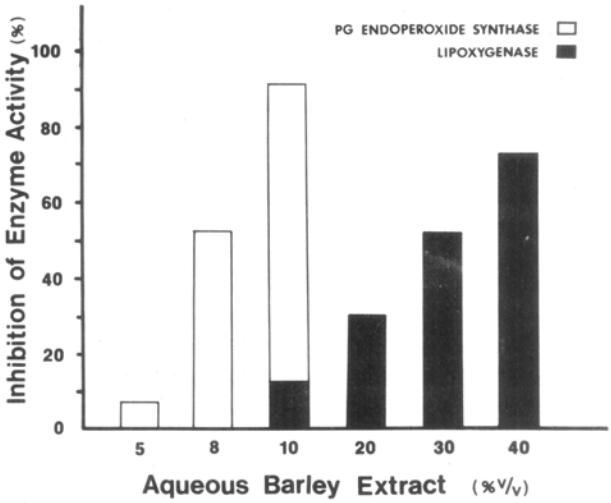

Fig. 1. Inhibition of PG endoperoxide synthase and lipoxygenase by aqueous extract of Barley. Different amounts of barley extract were incubated with enxymes using methods as described in the text.

A total of 14 fractions were obtained after sequential fractionation with petroleum ether, chloroform, ethylacetate and acetone 10% (v/v) of each fraction was used to assess the effect on the two enzymes. Table 1 shows an outline of inhibitory action of various fractions on PG endoperoxide synthase and lipoxygenase. Inhibition of endoperoxide synthase by 99% was exhibited by fractions No. 13 and 14 respectively (Fig. 2). On the other hand, fractions No. 8, 9 and 12 inhibited lipoxygenase by 62, 48 and 52% respectively (Fig. 2).

It is evident from our data that 5 times less amount of *barley* extract is required for 100% inhibition of PG endoperoxide synthase than needed for 70% inhibition of lipoxygenase (Fig. I). This was further confirmed from sequential fractionation of barley. Almost 100% inhibition of PG endoperoxide synthase was obtained by fractions No. 13 and 14 while maximum inhibition achieved for lipoxygenase, by fraction No. 8, was *62%* (Fig. 2). It is noteworthy that fraction No. 14, which was a starchy leftover, strongly inhibited PG endoperoxide synthase. These observations suggest that barley extract contains inhibitor(s) that alter AA metabolism. This inhibitor may function as a blocker of PG endoperoxide synthase more effectively than that of lipoxygenase.

At this stage, it is difficult to comment on chemical nature of these indigenous inhibitor(s), however, they showed significant water solubility and heat-stability.

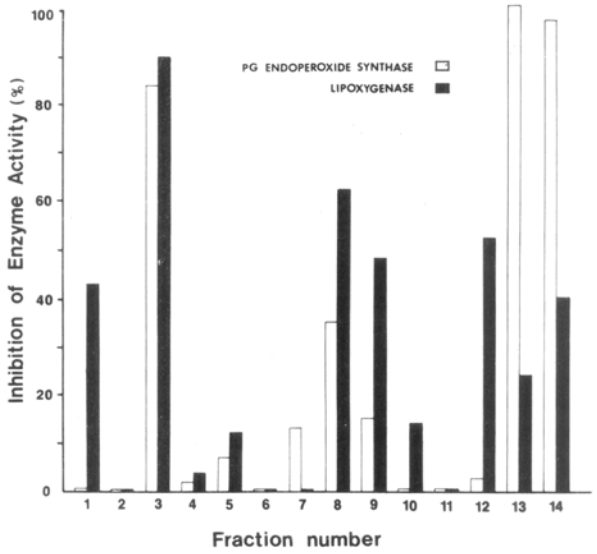

Fig. 2. Fractionation of aquous extract of barley by organic solvents: Fractions were obtained by extracting BE (Aq) in petroleum ether, chloroform, ethyl acetate and acetone using methods as described in the text.

Table 1. Effect of barley fractions on AA metabolism

Fraction #	PG Endoperoxide Synthase	Lipoxygenase
1.	0	++
2.	0	0
3.	++++	++++
4.	0	0
5.	0	0
6.	0	0
7.	0	0
8.	++	+++
9.	0	++
10.	a	+
I1.	0	0
12.	0	+++
13.	++++	+
14.	++++	++

Further purification and characterization of these inhibitors will reveal the true nature of these substances. They may he used as inhibitors of AA metabolism or each inhibitor may be applied individually to inhibit the specific enzyme.

References

Botdia, A. (1978). Atherosclerosis. 30, 355-360.
Encylcopedia Americana. (1985). 3, 243-244.
Encyclopedia Britannica. (Micropedia), (1980). 1. 821.
Hamberg, M,, Sevensson, J. and Samuelsson, B. (1975). Proc. Natl. Acad. Sci. USA. 72,2994.
Hammerstrom, S., and Diezfalnsy, U. (1980). Adv. in Prostag and Thrombox. Res. (Samuelson, B., Ramwell, P .W. (eds) 6, 267.
Saeed, S.A., Butt, N .M., McDonald-Gibson, W '.J., and Collier, H.O.J. (1981). Biochem. Soc. Trans., 9, 444.
Srivastava, N .C. (1984). Biomed. Biochim. Acta_ 43, 335-336.
Srivastava, K.C. (1984). Prostag Leuk and Med. 13(2), 227-235.