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THE INHIBITORY EFFECT OF INTERMEDEOL ON SOYBEAN LIPOXYGENASE AND ITS CYTOTOXICITY

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İNTERMEDEOLÜN SOYA FASULYESİ LİPOOKSİSENAZI ÜZERİNE İNHİBİTOR ETKİSİ VE SİTOTOKSİSİTESİ

Özet: *Abies nordmanniana ssp. bornmuelleriana'nın yapraklarından kloroformla ekstraksiyon yapılarak intermedeol [(4S, 5S, 7R, 10S)-eudesm-11-en 4-ol] izole edildi ve UV, IR, 1H-NMR and C-NMR, [a]D20 +15.4 (c 6.05, CHCl3) spektroskopik yöntemleri kullanılarak yapısı aydınlandı.İlave olarak intermedeolün soya fasülyesi lipoxygenazı (linoleate: oksijen oksidoredüktaz, EC 1.13.11.12) inhibe ettiği ve IC50 değerinin 42.5 μM olduğu saptandı. Aktivite ölçümlerinde substrat olarak linoleik asit kullanıldı. Intermedeolun toksisitesi, tuzlu su karidesi (Artemia salina) kullanılarak yapılan letalite testi ile saptandı ve LC50 değerinin 31 ppm olduğu bulundu. Toksik etki, kanser hücreleri ile yapılan sitotoksite bulguları ile korelasyon göstermektedir. Araşidonik asit metabolizması sonucunda oluşan metabolitler çeşitli hastalıklara neden olduğundan bu metabolik yolun kontol basamaklarını katalize eden enzimlerin inhibisyonunun tıbbi önemi vardır.*

Anahtar Kelimeler: *Abies nordmranniana ssp. bornmuelleriana, İntermedeol, Tuzlu su karidesi, Soya fasulyesi lipoksigenaz inhibisyonu.*

Summary: *Intermedeol [(4S, 5S, 7Rr, 10S)-eudesm-11-en 4-ol] was isolated from the chloroform extract of the leaves of Abies nordmanniana ssp. bornmuelleriana and identified by spectroscopic methods (UV, IR, 1H-NMR and 13C-NMR [a]D20 +15.4 (c 6.05, CHCl3). Additionally, it was found that intermedeol is an inhibitor of soybean lipoxygenase (linoleate: oxygen oxidoreductase, EC 1.13.11.12) with an IC50=42.5 μM. Activities were measured by using linoleic acid as substrate. Toxicity of the intermedeol was determined by means of the brine shrimp (Artemia salina) lethality assay and LC50 was found to be 31 ppm. The results well correlated with the cytotoxicity in cancer lines. Since, lipoxygenases catalysis the key steps in the conversion of arachidonic acid to the mediators which are the causes of a variety of diseases, the inhibition of the enzymes have a very important medical signifircance*

Key Words: *Abies nordmannianra ssp. bornmuelleriana, Intermedeol, Brine shrimp, Soybean lipoxygenase inhibition.*

INTRODUCTION

Lipoxygenases are the enzymes widespred in both plants and animals (1,2) and they catalyse the oxygenation of unsaturated fatty acids. The products are fatty acid hydroperoxides (3). The products of lipoxygenases are involved in the biosynthesis of various bioregulators(4). The metabolism of arachidonic acid by the lipoxygenase pathway generates leukotrienes(5) which have been implicated in the pathogenesis of a variety of human diseases such as artheroscloris, asthma and cancer (6). Multiple interaction of lipoxygenases generated compounds which can regulate specific cellular responses in inflammation and immunity (7). Recently, inhibition studies on both soybean and human lipoxygenase revealed the presence of an allosteric site that binds both substrate, linoleic acid and inhibitors (6).

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In view of biological activity and the medical significance of lipoxygenases, we tried to find out the selective inhibitors of the enzyme. Previously it has been reported that abietic acid, (-)-epicatechin-3-o-gallate, and (-)-epigallocatechin-3-o-gallate were the inhibitors of lipoxygenase activity (8,9).

Continuing our study on the inhibition of lipoxygenase activity we studied with intermedeol (a sesquiterpene compound from Abies nordmanniana ssp. bornmuelleriana) and found that it was also a lipoxygenase inhibitor. Furthermore, we tested its toxicity by brine shrimp lethality test. On the other hand, this study is the first attempt to use intermedeol on brine shrimp lethality test and determination of IC_{50} value of soybean lipoxygenase

MATERIALS AND METHOD

Plant Material: Abies nordmanniana ssp. bornmuelleriana (Pineaceae), was collected from Kızılcahamam, Ankara in September, 1992, and identified by D. Ercil (Hacettepe University). The voucher specimens have been deposited in the Herbarium at the Pharmacognosy Department, Faculty of Pharmacy, Hacettepe University, Ankara, Turkey (HUEF 92203).

Isolation: Intermedeol [(4S, 5S, 7R, 10S)-eudesm-11-en 4-ol] was isolated from the chloroform extracts of the leaves of Abies nordmanniana ssp. bornmuelleriana and identified by spectroscopic methods (UV, IR, 1H-NMR and 13C-NMR, [a]D20 +15.4 (c 6.05, CHCl3) (10).

Chemicals And Biological Material: Lipoxygenase type 5 from soybean and linoleic acid were purchased from Sigma Chemical Co. (St. Louis, U.S.A.). Artemia salina and Artemia salt were purchased from Hobby, Auf der Kiserführ 39,53127 (Bonn, Germany) and Hobby Dohse Aquaristic, (Bonn, Germany) respectively. All chemicals used were of analytical grade.

Enzymatic Assay: Soybean lipoxygenase activity was determined spectrophotometricaly by recording the formation of conjugated hydroperoxides from linoleic acid at 234 nm. Activities were measured by using LKB Ultraspec Plus Spectrophotometer (4054 UV/visible). The assay mixture contained 80 mM linoleic acid and sufficient amount of soybean lipoxygenase in 63 mM Borate/NaOH buffer, pH 9.0. The reaction was started by addition of enzyme and the reaction was followed at 25°C. The activities were measured for 40 seconds. The reaction was linear during this period. All assays were performed three or four times.

One unit (U) of activity is the amount of enzyme which catalyzes the formation of 1 mmol of hydroperoxy linoleate per min under the assay conditions. E234 of 25,000 mol-1/liter cm-1 was used for the calculations (11).

CYTOTOXİC ACTİVİTYTEST:

Hatching the shrimp: Brine shrimp eggs were hatched in a dish containing artificial sea water which was prepared with a commercial salt mixture 3.8 g/l and double amount of distilled water. A special dish with two compartment having one side darkened and the other illuminated was used. The eggs (ca. 50 mg) were sprinkled into the darkened large compartment. After 48 hours the phototropic nauplii were collected by pipette from large lighted sight.

Bioassays: Ten shrimps were transferred to each sample with capillary and artificiale seawater was added to make 5 ml. The nauplii were left under a flourescent lamp (36W) and countered after 48 h of illumination. After 48 h, % deaths in controls and each dose of sample were determined (12). Authentic substance gallic acid (Riedel) has a LC50= 4.03 (ppm) (95 % confidence limits 6.93-2.33).

LC_{50} determination: The data were analysed with the FINNEY (probit analysis method) computer program (DOS) to determine LC50 values and 95 % confidence intervals (The Finney computer program

was obtained from Prof. McLaughlin, (Purdue University, USA).

RESULTS AND DISCUSSION

Lipoxygenase is one of the key enzymes in the biosynthetic cascade leading to arachidonic acid to important mediators. Selective enzyme inhibitors are required for studies on the biochemical and physiological actions of different arachidonic acid metabolits. Furthermore, inhibitors affecting the arachidonic acid pathway should be useful for the therapy of various diseases. Many lipoxygenase inhibitors are currently being studied for use in the treatment of cancer and asthma (13-16). Many compounds were potent inhibitors of 5-lipoxygenase (14,16).

The lipoxygenase reaction velocity was found to be strongly dependent on substrate concentration. At higher substrate concentration an inhibitory effect was observed (17). Therefore we also investigated the inhibitory effect of substrate at high concentration in our previous report (8). At 80 mM linoleic acid concentration the initial velocity was maximal and substrate inhibition was not observed. So, in the present study using 80 mM linoleic acid, the enzyme activities were measured at different concentrations of intermedeol, between 10-80 mM. The steric structure of intermedeol isolated and used in the study is given in Figure 1. Intermedeol showed significant inhibitory effect against soybean lipoxygenase with an IC50 value: 42.5μM (Figure 2).

In vitro assays were used to screen the cytotoxic activity of intermedeol in brine shrimps to determine the concentrations (μg/ml) which cause 50% lethality (LC50). Toxicity was determined by means of the brine shrimp (A. salina) lethality assay, which gives result that correlate well cytotoxicity in cancer cell lines such as KB, P-388, L5178Y and L1210 (18). In this study, we showed that intermedeol has moderate effect (LC50=31 ppm) in the brine shrimp lethality bioassay. Intermedeol is a soybean lipoxygenase inhibitor which may inhibit human lipoxygenases. Understanding how - intermedeol inhibits lipoxygenase may help in the development of novel anti-cancer drugs used for the treatment where lipoxygenases are involved.

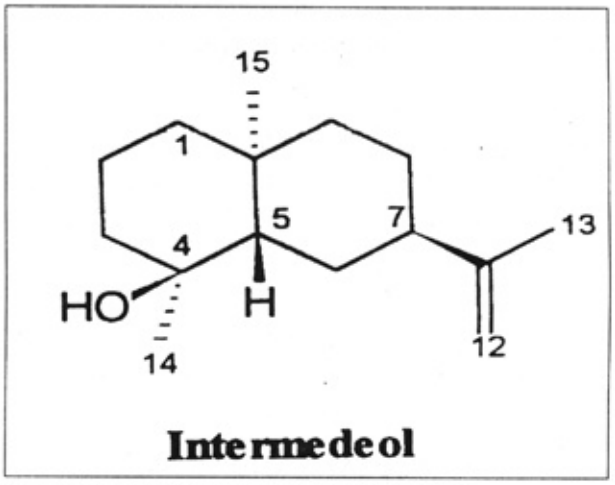

Figure 1. The chemical structure of intermedeol.

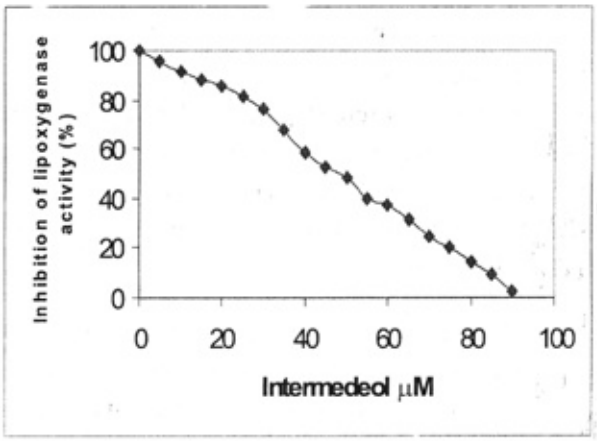

Figure 2. Inhibition of soybean lipoxygenase by intermedeol. The IC_{50} value was determined to be 42.5 μM.

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