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# Bovine Lens Aldose Reductase Inhibitory Effects of Some Natural Monoterpenes

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**Abstract** □ Carvomethone, (+)pulegone, (−) isopulegol and (−)menthone, which are natural and widely distributed in the plantkingdom, were examined for their Lens Aldose Reductase inhibitory effects. All monoterpenes tested showed the the mild inhibitory effects. Inhibition percents of four monoterpenes were in the range of 23~42% at $10^{-3}$M and 5~21% at $10^{-4}$M.

**Keywords** □ Monoterpenes, Carvomethone, (+)Pulegone, (−)Isopulegol, (−)Menthone, Bovine Lens Aldose Reductase.

Three types of cataracts are seen among diabetics. The first one is the metabolic cataract, which is the classical opacity occuring primarily in juvenile diabetics and significantly correlated with uncontrolled diabetes. The second one is the senile cataract, which is due to sclerosis of the lens nucleus and the most common one observed in adult diabetics. The third one is the cataracta complicata, which is also called secondary cataract, related to intraocular disease.

It is generally well known that activity of lens aldose reductase (LAR), an allosteric enzyme of lens sorbitol pathway, is greatly enhanced in the diabetic hyperglyceimic state.

Although insulin deficiency can be ameliorated by diet, insulin injection, or oral hypoglycemic agent therapy, standard treatment has not been able to prevent the development of chronic complications affecting the eyes, kidneys, nerves and arteries[1].

In the lens, the enzyme aldose reductase reduces aldoses such as glucose and galactose to their corresponding sugar alcohols. Elevated amounts of glucose in the deabetic lens would result in an intracellular accumulation of sorbitol, because polyols do not readily diffuse across cell membranes[2-8].

It is proposed that intracellular polyol accumulation causes osmotic swelling and eventual disruption of cell architecture. The strongest evidence in favor of this hypothesis is the finding that several drugs that inhibit aldose reductase significantly retard cataract formation in diabetic and galactocemic rats[9]. A number of specific inhibitors of aldose reductase have been described in the literature and they are important in clearing the relationship of sorbitol accumulation to the formation of diabetic and galactocemic manifestations, and may be of possible future clinical use[10-14] monoterpenes, which are utilized in food and cosmetic industry and widely distributed in plant-kingdom, were not yet assayed for their effects on the lens-aldose reductase activity.

On the basis of above mentioned considerations four natural monoterpenes were examined for their effects on the bovine LAR-activity.

## EXPERIMENTAL METHODS

Bovine eyes were obtained from a local

abattoir soon after slaughtering and the lenses were removed and frozen until used. Cellophane seamless tubing 18/32 inch for dialysis was purchased from E. Merck. The biochemicals were purchased from the following sources; NADPH (Tokyo Chem. Co.), DL-glyceraldehyde (Sigma Co.), (+)Pulegone (Fluka AG), (−)Isopulegol (Fluka AG), (−)Menthone (Fluka AG) and Carvomenthone (Fluka AG)

*Preparation of Enzyme*

All the operations were performed in a cold room at 4°C. Lenses (60g) were homogenized in 300 ml of cold 5 mM phosphate buffer, pH 7.4 and centrifuged at 18,000×g for 15 minutes to remove insoluble materials. 40% ammonium sulphate was added to the supernatant. After the thick suspension had been allowed to stand with occasional stirring for 15 minutes to ensure completeness of precipitate was discarded. Aldose reductase was then precipitated from the 40% supernatant solution by the addition of ammonium sulphate to 75% saturation and was recovered by centrifugation. The preciprtated enzyme was redissolved in 5 mM phosphate buffer, and dialyzed 3×4 hours against 10 volumes of phosphate buffer. A DEAE-cellalose column (2×30 cm) was previously equilibriated with 5 mM phosphate buffer. The dialyzed enzyme preparation was absorbed on the column, and the column was washed with 5 mM phosphate buffer until the absorbance at 280 nm of the eluate was less than 0.1, the elution of enzyme was accomplished with a linear gradient.

*Determination of Enzyme Activities*

The reaction mixture contained: 0.1 M phosphate buffer, pH 6.2; NADPH, $2.5\times10^{-4}$M; DL-glyceraldehyde, $1.5\times10^{-3}$M; and the enzyme. The total volume of this reaction mixture was adjusted to 1 ml. The reference blank consisted of all the above compounds except the substrate. The effect of inhibitors on the enzyme activities was determined by including in the reaction mixture the compounds being tested at the desired concentrations. The reaction was carried out at 25° C and initiated by the addition of substrate. A unit of activity was defined as a change in absorbance of 0.001 unit per minute.

## RESULTS AND DISCUSSION

Monoterpenes are widely distributed in the edible plants and many synthetic trials have been reported. But the effects of monoterpene derivatives on the lens aldose reductase activity have not been reported in the literature.

On the basis of this consideration we started to investigate the effects of monoterpenes on the activity of the bovine LAR. Monoterpenes tested in this experiment showed mild inhibitory effects on the bovine LAR activity.

As shown in Table 1 inhibition percents of four monoterpenes were in the range of 23∼42% at $10^{-3}$M and 5∼21% at $10^{-4}$M. Among them most inhibitory effects was found in the case of (+) pulegone and least effect in the case of (−) menthone.

These mild LAR inhibitory effects of monoterpenes tested could not be construed as an applicable substances for the individual medicopharmaceutical use. But these results stimulate us to carry the further studies on the additive

**Table 1. Effects of some monoterpenes on Lens Aldose Reductase activity**

| Name | Inhibition(%) | |
|---|---|---|
| | $10^{-3}$M | $10^{-4}$M |
| Carvomenthone | 37 | 21 |
| (+)Pulegone | 42 | 20 |
| (−)Isopulegol | 25 | 17 |
| (−)Menthone | 23 | 5 |

and synergetic effects of monoterpene-mixtures in the edible plants and medicinal plants and to consider the applicability of the monoterpene containing herbs for the prevention and retardation of diabetic cataracts.

Further investigations on the LAR inhibitory effects of other monoterpenes and structure-activity relationships are in progress.

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