CHOLERETIC EFFECT OF *ARTEMISIA CAPILLARIS* EXTRACT IN RATS

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*Artemisia capillaris* THUNB. has long been used in China and Japan to treat jaundice. The extract of this plant was experimentally confirmed to possess a choleretic effect in dogs, rabbits or rats (1-3), and 6,7-dimethyl-esculetin (2, 3) and capillarisin (4) were isolated as effective constituents from the extract.

The mechanism for bile formation is not well known but three processes have been empirically proposed: bile acid dependent secretion, bile acid independent secretion and bile duct secretion (5). Bile acid dependent flow is referred to the slope of a regression line between bile acid secretion and bile flow, while bile acid independent flow is referred to as component on the ordinate when the regression line is extrapolated to the ordinate. The bile acid independent flow comprised bile acid independent secretion and bile duct secretion. Although such a single, linear regression analysis has been questioned because infusions of bile acids into the bile acid depleted animals showed a relationship in which the slope progressively declined as the biliary bile acid concentration increased (6), the analysis is yet applicable for primary assay to examine whether changes in bile flow are due to the bile acid secretion. The effects of compounds such as thyroid hormone, scillaren, hydrocortisone, phenobarbital, etc. were examined by the method. The previous experiments on *A. capillaris*, however, recorded bile flow after a single intravenous (1, 2) or intraduodenal (3) administration and the effect on bile acid secretion and bile acid metabolism remained undetermined. We, therefore, determined biliary constituents such as cholesterol, phospholipids and bile acids in rats after multiple oral administration with the extract of *A. capillaris* for 4 and 7 days and examined the relationship between bile flow and biliary bile acid secretion. In addition, we also determined the pool size, distribution and synthesis of bile acids in the control and the treated animals.

Male Wistar rats weighing 250–300 g were kept in an air conditioned room (25±1°C, 50–60% humidity) in the light 12 hr a day (8.00 a.m. to 8.00 p.m.) and maintained on a commercial balanced stock diet (Japan CLEA CA-1, Tokyo, Japan). Usually, four to five rats were housed in one cage but they were kept individually in metabolic cages during the experimental periods when feces were collected.

Sixty grams of dried flowers of *A. capillaris* THUNB. were extracted with 3 L of water for 1 hr on a water bath and filtered. The filtrate was concentrated to 90 ml under reduced pressure. A dose of 3 ml of the extract, equivalent to 2 g of the dry material, was administered orally once a day at 9.00 a.m. for 4 and 7 days. Control rats were given the same volume of the vehicle.

In the first series of experiments, the rats were anesthetized with sodium pentobarbital (50 mg/kg, i.p.) one hour after the last administration and the bile duct was can-
nulated with PE-10 polyethylene tubing to collect bile for 30 min. The rectal temperature was maintained at 36–37°C using an electric warm plate. Next, blood was withdrawn by puncture of the heart and liver, and small and large intestines with their contents were removed. In the second series of experiments, the bile duct was cannulated one hour after the administration and the bile was collected at 30 min, 1, 2 and 3 hr under sodium pentobarbital anesthesia. In this experiment, physiological saline was given i.p. at 1 and 2 hr in volumes corresponding to the bile secreted. The liver and the small and large intestines together with their contents were homogenized with distilled water and portions of the homogenates were lyophilized.

Biliary bile acids, cholesterol and phospholipids, and fecal and tissue bile acids were determined, as reported previously (7–9). The pool size of bile acids was calculated by summing the amounts in the bile, liver and intestines. Since the fecal excretion rate of bile acids was presumed to correspond to the hepatic synthetic rate in a steady state, such was used for the value of synthesis. Turnover frequency was calculated by dividing the amount of secretion by the pool size.

One hour after the last of four or seven successive administrations of the extract of A. capillaris, bile flow markedly increased but biliary secretion of cholesterol, phospholipids and bile acids remained unchanged. The composition of bile acids was not affected. Duration of the choleresis was about 6 to 8 hr in another experiment in which the rats were individually kept in Bouleman cages and the bile flow was examined up to 24 hr after the last administration.

Figure 1 shows the relationship between bile flow (ordinate) and bile acid secretion (abscissa) in control and treated rats. From the relationships, the bile acid dependent bile flow was calculated to be 16.5 μl/mole in the control and 18.1 μl/mole in the treated, while the bile acid independent flow was 1.12 ml/hr per rat in the control and 1.80 ml/hr per rat in the treated. The bile acid dependent flow in the treated rats did not change but the bile acid independent flow increased to about 60% over the level in control rats.

The pool size distribution, secretion, synthesis and turnover frequency of bile acids after the treatment for 4 days are given in Table 1. In the control rats, the pool size was around 40 mg/rat and bile acids were mainly located in the small intestine. The secretion was about 50 mg/hr per rat and the synthesis was 11 mg/day per rat. The extract of A. capillaris slightly increased the secretion and the turnover frequency but the increases were statistically insignificant (P>0.05).

These data showed that the extract of A. capillaris increased bile flow by increasing the bile acid independent bile flow but affected neither the enterohepatic circulation nor the metabolism of bile acids even after multiple administration for 4 days. Therefore, the extract may increase bile flow by
stimulating the activity of the membrane Na+,K+-ATPase, which is postulated to be closely related to the bile acid independent bile formation (10, 11), or by stimulating secretin secretion which increased ductal bile secretion (12), or by organic anionic choleresis (13) produced by the constituents of the extract. Mashimo et al. (2) observed that two metabolites, 6-hydroxy-7-methoxy and 6-methoxy-7-hydroxycoumarin, of 6,7-dimethylesculetin were excreted into the bile. The choleretic effect of the extract appeared 90 min after the oral administration and lasted for 6 to 8 hr. During this period, about 3 ml of bile was secreted as induced by the extract of 2 g of the dry material. If such an amount of water is secreted by osmotic force, about 0.5 mmole of solutes is required. The major constituent of A. capillaris is 6,7-dimethylesculetin and its content was only 0.6-1.5% in our material. Even if the other constituents such as capillarin, capillarisin, capillene and capillin are taken into account, the total constituents amount to only 2% at most, roughly 0.2 mmole per 2 g of the dry material. If we assume that all the constituents are absorbed and secreted into bile, such are not sufficient to produce 3 ml of water secretion. Therefore, other mechanisms are probably involved in the choleretic effect of A. capillaris, in addition to the drug-induced osmotic choleresis, and/or other unknown choleretic compounds are included in A. capillaris.

**REFERENCES**


