CHOLINOMIMETIC EFFECTS OF AQUEOUS EXTRACTS FROM *Carum copticum* SEEDS

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An aqueous extract from roasted seeds of *Carum copticum* (omum) has cholinomimetic effects. It shows muscarinic effects on rabbit duodenum, guinea-pig ileum and rat jejunum, and on the blood pressure of rat and cat. These effects are blocked by atropine. It also has a nicotinic action on the frog rectus preparation and atropinized cat blood pressure. Its effect is potentiated by physostigmine and antagonized by cholinesterase or alkalinization. Paper and gas chromatography have confirmed the presence of acetylcholine and choline in the roasted omum seed extract.

Aqueous extracts from seeds of *Carum copticum* (Benth.) f. Umbelliferae (commonly known as omum or Bishop's weed) are used in household remedies and also as a spice in food in India. A watery extract of omum seeds is used to relieve gripe in children. In diarrhoea, either roasted omum seeds are taken, or a watery extract made from them is given as a draught. In the present study the pharmacological effects of cold or hot aqueous extracts of whole or ground seeds of *Carum copticum* were investigated.

Methods

Preparation of omum extracts Aqueous extracts were made by leaving the seeds of *Carum copticum* (10 g to 100 ml) either in cold or hot water for a period of 4 hours. These were designated as follows: (1) extract from whole seeds soaked in cold water (at room temperature of about 25° C) (OE 1); (2) extract from powdered seeds soaked in cold water (OE 2); (3) infusion with whole seeds (OE 3); (4) whole seeds roasted to dark brownish hue in a glass pan, added to water and left (OE 4). The fourth procedure is the one adopted commonly in households for preparing a draught against diarrhoea.

Biological procedures Isolated tissues including rat stomach (according to Vane, 1957), duodenum, jejunum, ileum and colon, and also guinea-pig ileum were each mounted in a 12 ml bath containing Tyrode solution kept at 37° C. Rabbit duodenum was kept in Ringer-Locke solution. The contractions of the tissues (magnified 3 or 4 times) were recorded with an isotonic lever. Carotid blood pressure was recorded in anaesthetized rats with a Condon's manometer according to Straughan (1958), and in cats under pentobarbitone anaesthesia with a standard mercury manometer. Drugs were administered into the cannulated femoral vein.

Paper chromatography Roasted omum seeds (100 g) were soaked in 800 ml water for 4 hours. The infusion was filtered and freeze-dried. One gram of the freeze-dried powder was extracted with 2 volumes of reagent grade absolute ethanol and filtered. The ethanol was removed under vacuum. The residue was taken up in 0.5 ml ethanol, from which 100 μl was spotted on Whatman No. 1 filter paper. Acetylcholine 100 μg in 100 μl was also spotted. Single dimension ascending chromatography was carried out with the solvent system described by Augustinsson & Grahn (1953) for choline esters. The chromatogram was developed with phosphomolybdic acid and stannous chloride. The paper chromatographic studies were performed by one of us (P.S.V.R.) at the National Institute for Nutrition, Hyderabad, A.P., India.

Gas chromatography The same freeze-dried extract of OE 4 was used as described above. The analysis of the extract by gas chromatography for the presence of acetylcholine and related substances was by the method of Jenden & Hanin (1974). Different amounts of OE 4 freeze-dried extract (48, 100, 191 and 451 mg) were each dissolved in 2.5 ml ammonium acetate buffer at pH 3.92. Valeryl choline (20 nmol) was added as internal standard to each sample. Tetraethyl ammonium chloride (final concentration 0.1 mM) was added as coprecipitant and choline and its esters were precipitated as the reineckate salts. After drying the precipitate, choline and its esters

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were converted to the corresponding tosylate salts. Choline was esterified to propionylcholine in the presence of propionyl chloride in acetonitrile. The samples were next subjected to demethylation in the presence of sodium benzenethiolate and the resultant tertiary amino analogues of choline esters were extracted into chloroform and injected into the gas chromatograph. Concentrations of choline and acetylcholine were calculated with reference to the added internal standard valeryl choline. Samples were injected into a gas chromatograph (Packard 7401) equipped with a flame ionization detector. The gas chromatographic studies were performed by one of us (I.H.) at the Department of Psychiatry, University of Pittsburgh School of Medicine, Pittsburgh, Pennsylvania, U.S.A.

Results

Effects on rat alimentary tract The four different aqueous extracts from omum seeds OE 1 to 4 were tested on rat isolated fundus, duodenum, jejunum, ileum and colon; the rat fundus was stimulated by all the extracts, OE 4 being the most potent. The extract OE 4 stimulated all parts of the rat alimentary tract. The cold extracts OE 1 and 2 either abolished the spontaneous activity of the duodenum or relaxed the tone of the jejunum, ileum and colon. The stimulant effects of OE 4 were studied further.

Cholinomimetic effects The stimulant effects of OE 4 on the guinea-pig ileum, rabbit duodenum and rat jejunum were blocked by atropine to the same extent as the stimulant effects of equiactive doses of acetylcholine. The depressant muscarinic effects of acetylcholine on rat or cat blood pressure were also mimicked by OE 4 and these depressor responses were abolished by atropine.

Omum extract 4 exerted acetylcholine-like effects at the neuromuscular junction and at autonomic ganglia. It caused a contracture of the isolated frog rectus muscle which was inhibited by (+)-tubocurarine. On cat blood pressure following administration of atropine (1 mg), the depressor responses to small doses of acetylcholine (200 ng) and OE 4 (0.2 ml 10%) were abolished, but larger doses of acetylcholine (500 μg) and OE 4 (5 ml 20%) produced a pressor response. The stimulant effect of OE 4 on the frog rectus preparation was potentiated when the tissue was exposed to physostigmine 10 μg/ml for 15 min before the application of OE 4, but no response was obtained with OE 4 previously incubated with dog serum for 5 minutes. A response was obtained, however, if the serum was first pretreated with an anticholinesterase, physostigmine 25 μg/ml, for 10 minutes. Similar results were obtained when dog RBC cholinesterase was used instead of serum. The effects of OE 4 on the preparation were abolished when the extract was taken to pH 10 by addition of NaOH 1 N, kept in a boiling water bath for 30 min, neutralized with HCl to pH 6, and then tested. These results all suggest the presence of an acetylcholine-like ester in the extract OE 4.

Paper chromatography Omum extract (100 μl) and standard acetylcholine were subjected simultaneously to ascending paper chromatography for 20 h as described in the methods section. The R_F value of blue spots developed with phosphomolybdic acid and stannous chloride with the test solution was 0.50 and this corresponds with the R_F value of acetylcholine standard 0.49.

Gas chromatography The gas chromatographic analysis revealed that OE 4 extract contains both choline and acetylcholine. No other esters of choline were found. The concentration of acetylcholine per gram of freeze-dried extract calculated from four estimates of different amounts of the OE 4 powder is 93.3 ± 7.6 (mean ± s.e.) nmol/g or 13.44 μg/g. This compares favourably with its biological activity in terms of acetylcholine which was 14.4 μg/g of the powder. The concentration of choline was 4626.7 ± 281.5 (mean ± s.e.) nmol/g or 481.19 μg/g of the freeze-dried extract.

Discussion The chromatographic results indicate that the biological activity of the extract (OE 4) from roasted seeds is largely due to acetylcholine. The presence of large amounts of choline in the seeds and the presence of acetylcholine only in the hot extracts suggests that acetylcholine is somehow formed during the process of roasting the seeds.

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