

The effect of cyclic monoterpene menthol on blood glucose, water and electrolyte excretion in rats

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Rowachol (Rowa Ltd., Bantry, Eire), a proprietary choleric containing the purified mono- and bicyclic monoterpenes menthol (32% w/v), pinene (17% w/v), menthone (6% w/v), borneol (5% w/v), camphene (5% w/v), and cineole (2% W/V) in olive oil, has been shown to cause dissolution of cholesterol gallstones in man and to inhibit hepatic HMG-CoA reductase in rats and man when administered *in vivo*. The present study was therefore designed to investigate the effect of menthol on water, electrolyte excretion and blood glucose concentration in rats. Menthol was administered in olive oil by gastric tube in a volume of 2ml/kg. In determining the dose-response relation for menthol, it was given at 1.0, 3.0 and 6.0 mmol/kg of body weight in a volume of 2 ml/kg. Water together with electrolyte excretion and blood glucose concentration were investigated using flame photometric and colorimetric techniques in rats (n= 48). The treatment of rats with menthol resulted in a decrease in blood glucose concentration (73.0 ± 1.39 , 67.5 ± 1.41 and 63.9 ± 2.06 mg/dl) that was significant ($p < 0.05$) in all the menthol treated animals (1.0, 3.0 and 6.0 mmol/kg) compared to that of the olive oil treated control group (75.4 ± 1.10 mg/dl). There was a significant dose ($p < 0.05$) dependent increase in urine Na^+ ion level (420 ± 15.00 , 450 ± 8.86 , 480 ± 10.52 mmol) and urine output volume (150 ± 5.67 , 200 ± 9.82 , 280 ± 11.02 ml) in menthol treated animals compared to their control groups 400 ± 12.82 mmol and 135 ± 6.55 ml respectively, while urinary K^+ excretion showed no significant change ($p > 0.05$) in all the treated animal groups (86 ± 2.08 , 87 ± 2.38 , 89 ± 2.09 mmol) with respect to the control (81.5 ± 1.75 mmol). The study indicates that menthol increases water intake, urine output and urine Na^+ excretion, and decreases blood glucose concentration and has no significant effect on urine K^+ excretion.

Key words: Menthol, monoterpenes, blood glucose, electrolyte, excretion.

INTRODUCTION

Plants are one of the most important resources of human foods and medicines. Rapidly increasing knowledge on nutrition, medicine, and plant biotechnology has revolutionized dramatically changed the concepts about food, health and agriculture. Nutritional therapy and phytotherapy have emerged as new concepts in healing systems which have quickly and widely been spreading in recent years [1]. Strong recommendations for consumption of nutraceuticals, natural plant foods, and

the use of nutritional therapy and phytotherapy have become progressively popular to improve health, and to prevent and treat diseases. Synthetic drugs and pharmaceuticals based on extensive safety, efficacy, mechanistic and clinical studies have significantly contributed to improvement of overall human health. However, a large number of these drugs have been withdrawn from the marketplace due to various toxicities and other adverse effects [2].

The peppermint herb has been used medicinally for thousands of years. Peppermint (*Mentha piperita*) is known in herbal, homeopathic and conventional medical practice to have aromatic, stimulant, stomachic, carminative,

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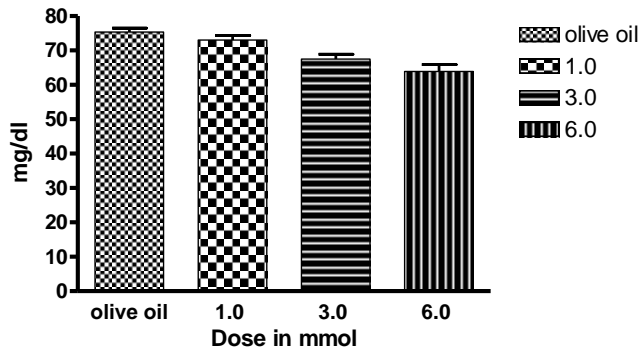


Figure 1. Effect of Menthol on blood glucose (mg/dl).

rubefacient and short lived local anesthetic effects. Kloss describes peppermint as an excellent remedy for chills, colic, fevers, dizziness, flatulence, nausea, vomiting, diarrhoea, dysentery, cholera, heart trouble, palpitations of the heart, influenza and even hysteria [3]. The peppermint oil is used as flavouring for toothpaste, cough syrup, mouthwash, liqueurs, soap, perfumes and sauces. Menthol, a key active component of the peppermint oil has been used for a variety of health conditions such as nausea, indigestion, cold symptoms, headaches, muscle and nerve pain, and irritable bowel syndrome. Peppermint has been described as an excellent remedy for chills, colic, fevers, dizziness, flatulence, nausea, vomiting, diarrhoea, dysentery and heart trouble [4]. Rowachol, a proprietary choleric containing the purified mono- and bicyclic monoterpenes menthol (32%w/v), pinene (17% w/v), menthone (6%w/v), borneol (5%w/v), camphene (5%w/v) and cineole (2%w/v) in olive oil, has been shown to cause dissolution of cholesterol gallstone in man [5] and to inhibit hepatic HMG-CoA reductase in rats [6] and man [7] when administered *in vivo*. As part of effort to investigate some of the acclaimed effect of menthol, the present study was undertaken to evaluate the effect of menthol on blood glucose concentration, water and electrolyte excretion in rats and to assess their potential usefulness as food supplements or pharmaceutical raw material for drug formulation.

MATERIALS AND METHOD

Experimental Animals and Procedure

Male and female Wistar rats (200-230g) were purchased from Veterinary Research Institute, Vom Jos. The animals were acclimatized in standard cages in the laboratory for one week with 12 hour light and 12 hours dark cycle through guided interference to light admittance in place the animals were kept. Food (poultry feed) and water were available *ad libitum*. 48 Wistar rats were randomly divided into 4 groups. Group 1 (Control) received olive oil alone, while groups 2, 3 and 4 had a

solution of 1.0, 3.0 and 6.0 mmol/kg menthol respectively in olive oil orally using gastric tube for 7 days. The study was conducted in accordance with the Organization for Economic Development (OECD) guidelines on good laboratory practice [8].

Materials

Menthol (Rowa Ltd., Bantry, Eire), Olive oil, Serum glucose assay kit (Randox Laboratories)

Administration of Menthol to Rats

Menthol was administered in olive oil by gastric tube in a volume of 2ml/kg. In determining the dose-response relation for menthol, it was given at 1.0, 3.0 and 6.0 mmol/kg [9] of body weight in a volume of 2 ml/kg.

Effect of Menthol on blood glucose concentration.

Blood sample was collected through cardiac puncture after anaesthetizing the animals with urethane (0.6 ml/100g) and put into plain centrifuge tubes containing EDTA. Blood glucose concentration was assayed as described by the method of Trinder [10]. Absorbance of the serum was taken at 505 nm after 10 μ l of the serum sample mixed with 1ml of glucose oxidase reagent had been incubated at 37°C for 30 minutes.

Effect of Menthol on water and electrolyte excretion

Daily water consumed in each group was monitored and converted to volume per kilogram per 24 hours. Eight rats were randomly isolated from each group and daily urine was collected between 4.00 pm to 8.00 am for each rat. The volume obtained was converted to urine output (ml) per kilogram (kg) body weight per 24 hours, and average of the eight collections was taken as volume for the week. Sodium and potassium excretions were determined from the urine collected using flame photometer and recorded as mean of collections per 7 days.

Statistical Analysis

Data were expressed as the mean \pm (SEM). Data were analyzed by ANOVA) using Graph Pad prism 4. The differences in mean were considered statistically significant when $p \leq 0.05$.

RESULT

The treatment of rats with menthol resulted in a decrease in blood glucose concentration that was significant ($p > 0.05$) in the treated animals compared to that of the control group treated with olive oil as shown in Figure 1. Menthol resulted in a dose dependent increase in urine Na^+ ion and urine volume. This effect occurred through-

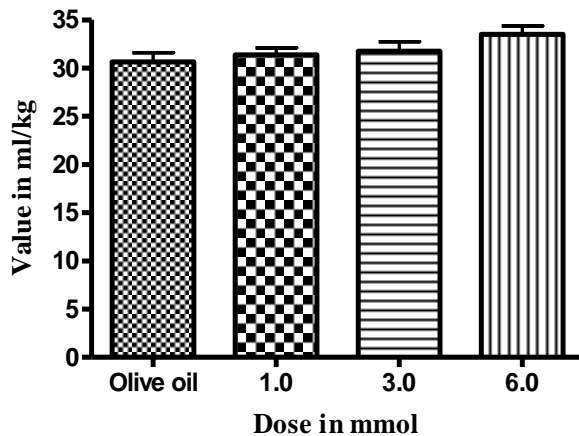


Figure 2. Effect of Menthol on water intake (ml/kg) after 7 days.

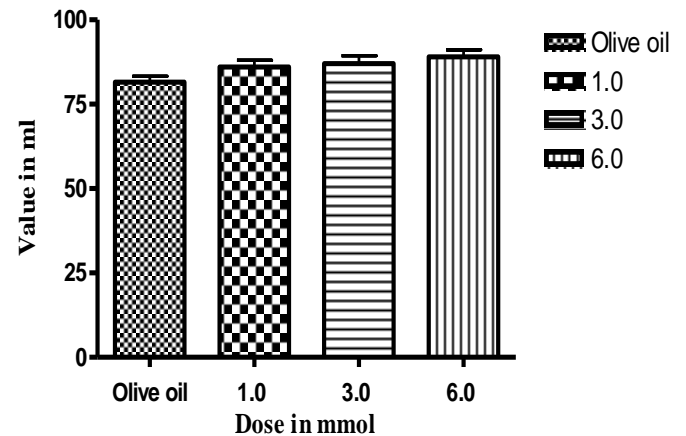


Figure 5. Effect of Menthol on K⁺ Excretion (mmol) after 7 days.

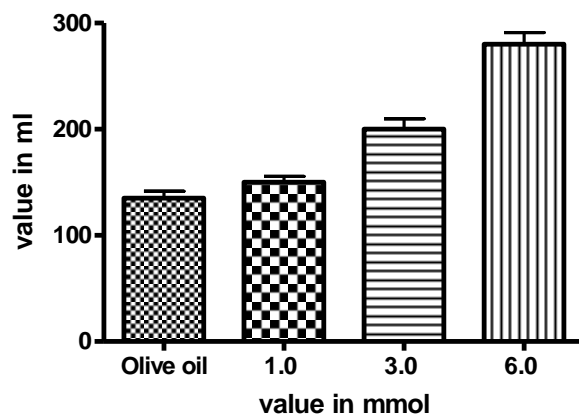


Figure 3. Effect of Menthol on Urine Output (ml) in 7 days.

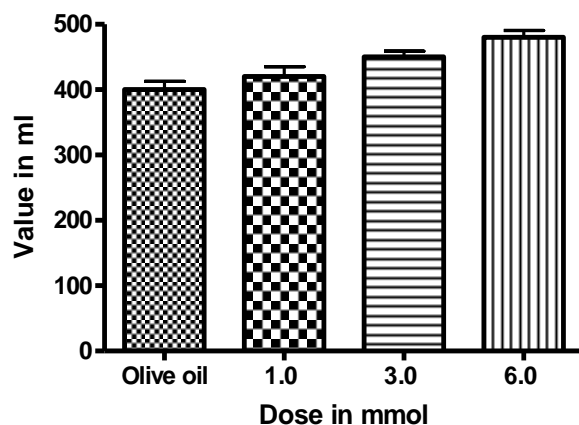


Figure 4. Effect of Menthol on Na⁺ Excretion (mmol) after 7 days.

out the duration of this study (Figures 2-5).

In Figure 5, Urinary K⁺ excretion showed no significant change ($p > 0.05$) in all the treated animal groups (86 ± 2.08 , 87 ± 2.38 , 89 ± 2.09 mmol) with respect to the control (81.5 ± 1.75 mmol).

DISCUSSION

The treatment of rats with menthol resulted in a decrease in blood glucose concentration that was significant in the treated animals compared to that of the control group (Figure 1). This decrease may have been due to increase in food digestion, thus increase in metabolism. Peppermint oil has been shown to stimulate gallbladder contraction, resulting in increased digestion through stimulation of the vagus nerve, which then leads to secretion of bile. Essential oils, such as peppermint exerts a significant smooth muscle relaxant effect believed to relate to inhibition of calcium channels [11]. Menthol is known to block the carbachol (acetylcholine-like) induced influx of calcium ions into cells. This calcium channel blocker action of menthol exerts pharmacological effects similar to those observed with current prescription medications such as nifedepine or diltiazem, which are calcium channel antagonists [12]. Calcium channel blocking drugs, such as nifedepine or diltiazem, are known to exert effects on upper gastrointestinal motor function, including inhibition of esophageal peristalsis, and a reduction of the lower esophageal sphincter pressure, in the absence of major effects on gastric motor function [12].

Treatment of rats in this study with menthol resulted in a dose dependent increase in urine Na⁺ ion and urine volume. This effect occurred throughout the duration of this study (Figures 2-5). This may be indicative of a diuretic effect of menthol in the rats, since water intake

was not significantly ($p < 0.05$) affected in all the groups. Classical diuretic such as furosemide is known to increase the urine output and sodium ion level in animals and human [13, 14]. This is an effect similar to that noticed in this study. It is known that the regulation of urine volume is by anti-diuretic hormone (ADH), atrial natriuretic hormone and aldosterone [15]. The increased urine volume may have been due to the effect of menthol in decreasing ADH and aldosterone. The increase in Na^+ secretion indicates decrease in the absorption of water in the distal and collecting tubules. Also the menthol may have cause an increase in secretion of atrial natriuretic hormone leading to loss of Na^+ in the urine. However, the non-significant increase in the Potassium ion level in the treated rats may be an indication that menthol may have inhibited the $\text{Na}^+ - \text{K}^+$ ion exchange mechanism at the distal tubule of the nephron, thus suggesting that menthol may in part be acting on the distal convoluted tubule [13].

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