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Original Article

Safety assessment of menaquinone-7 for use in human nutrition

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ABSTRACT

Vitamin K occurs widely in foods and has been shown to have a beneficial effect on the cardiovascular system, as well as anticancer, anti-inflammatory, and antiosteoporosis properties. A previous study indicates that long-chain menaquinone-7 may be more bioavailable than vitamin K and short-chain menaquinones. In the present study, acute, subacute toxicity and genotoxicity assays were carried out to evaluate the safety of oral menaquinone-7 in albino Wistar rats. Oral administration of menaquinone-7, at a concentration of 2000 mg/kg, did not cause toxic symptoms in either male or female rats. A subacute toxicity study also proved the safety and tolerance of prolonged treatment (for 90 days) with menaquinone-7 in rats, as evidenced by biochemical, hematological, and urine parameters as well as by histopathological analysis. Genotoxicity and mutagenicity studies were performed by comet, micronucleus, and Ames tests on *Salmonella typhimurium* strains, which showed cellular safety and nonmutagenicity of menaquinone-7. The results indicate the safety of menaquinone-7 for human consumption.

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1. Introduction

It was discovered over 80 years ago that food-derived components are essential factors in blood coagulation. In 1929, experimental studies at the University of Copenhagen using chicks fed a diet extremely low in fat revealed that low-fat-diet birds developed a hemorrhagic condition and their blood coagulation time was slower than that in the control group [1]. Further investigations showed that the deficiency of a fat-soluble vitamin, vitamin K, was responsible for the hemorrhagic tendency. Vitamin K occurs in two biologically active forms: phylloquinone (vitamin K1) and menaquinones (vitamin K2). Vitamin K1 is produced by green and leafy vegetables, and algae, whereas vitamin K2 is predominantly of microbial origin [2,3]. The major form of dietary vitamin K is considered to be K1, accounting for ~90% of total vitamin K intake [3]. Vitamin K2 is found in animal products, meat, dairy, eggs, and fermented foods such as cheese, yoghurt, and the traditional Japanese food natto (*Bacillus natto* fermented soya beans); vitamin K2 is also synthesized by intestinal microflora [4].

In addition to its role in the synthesis of hepatic blood-coagulation proteins, vitamin K has been found to play a role in bone health, cardiovascular health, prevention of cancer, suppression of inflammation, prevention of brain oxidative damage, sphingolipid synthesis, and osteoporosis [5–9]. Epidemiological studies from Japan and Europe suggest an association between poor vitamin K2 (but not vitamin K1) intake and increased postmenopausal bone loss, arterial calcification (notably in diabetes), end-stage renal disease, and cardiovascular disease during normal aging, as well as an increased risk of bone fracture [10,11]. Supplementation with vitamin K2 may prevent age-related bone loss and improve bone strength, arterial elasticity, and cardiovascular health [12–15]. Available information indicates that current intake of vitamin K, particularly K2, may not be sufficient for the maintenance of bone health and cardiovascular health [16,17].

Compared to phylloquinone, menaquinones, especially long-chain menaquinone-7, may be beneficial for bone and soft tissue, specifically for protein carboxylation and production. This is probably because menaquinone-7 is better absorbed and is more bioavailable than vitamin K1 or short-chain menaquinones such as menaquinone-4. Because there is a paucity of studies on the toxicity of menaquinones, especially long-chain menaquinones, we present in this paper a systematic toxicity and safety study of oral vitamin K2–7 in rodents.

2. Materials and methods

2.1. Chemicals

Vitamin K2–7 (MenaquinGold) was provided by Viridis Biopharma Pvt. Ltd. (Mumbai, India). All other chemicals used were commercially available in the purest form.

2.2. Animals

Male and female 7–8-week-old albino Wistar rats were obtained from the experimental animal facility at Gujarat Ayurved University, Jamnagar, India. Animals were housed in polypropylene cages under a controlled atmosphere (25 ± 1°C at 60–70% relative humidity) with a 12-hour light–12-hour dark cycle [18]. All animals were fed with Amrut brand rat pellet feed supplied by Pranav Agro Ltd. (Pune, India) and tap water *ad libitum*. Following at least 1 week of acclimatization, the rats were divided randomly into seven groups of eight animals each for experiments, as discussed below. Menaquinone-7 compound was suspended in a propylene glycol vehicle. Test formulations were administered to the animals through oral gavage. All procedures were in accordance with the guiding principles established by the Animal Care Committee of our University.

2.3. Oral acute toxicity

The toxicity study was conducted according to the modified the Organisation for Economic Co-operation and Development (OECD) protocol as practiced by the Institute for Post Graduate Teaching and Research in Ayurveda and was cleared by the Institutional Review Board (IRB) (IAEC-04/09-10/Proj-02) of Gujarat Ayurveda University, Udupi, India. Healthy, 10–12-week-old Albino Wistar rats ($n = 40$) of average weight (150–210 g) were randomly divided into five groups, with four male and four female rats in each group, and kept in separate cages. The animals were supplied *ad libitum* with water and pellet feed throughout the study, and subjected to overnight fasting prior to dosing with menaquinone-7 or placebo. The control group received propylene glycol vehicle, and each treated group received menaquinone-7, at doses of 0.5 mg/kg, 1.0 mg/kg, 10 mg/kg, or 20 mg/kg, once daily for 14 days. LD_{50} value was determined by administration of a single dose of 2000 mg/kg menaquinone-7 for 14 days. The animals were monitored for general behavior, toxic signs and symptoms, or mortality during the experimental period. At the end of the study, the animals were sacrificed by CO_2 asphyxiation and examined for gross changes in vital organs.

2.4. Ninety-day subacute oral toxicity in rats

2.4.1. Animal treatment

The protocol employed was a modified version of the OECD guideline 408. Ten animals per group, five male and five female Wistar Albino rats, were used instead of 10 male and 10 female rats as in the original OECD protocol. The animals were oral treated with 0.1 mg/kg, 0.5 mg/kg, and 1.0 mg/kg menaquinone-7 once daily for a period of 90 days. Animals of the control group received propylene glycol as the vehicle. Menaquinone-7 solution was prepared fresh every day and administered at a constant volume of 1 mL/100 g body weight between 8 AM and 9 AM. Behavior, mortality, and changes in body weight and food consumption were recorded. Upon completion of 90-day subchronic protocol sacrifice, autopsy of animals and histopathology of selected organs were performed. The number of experimental animals per group was approved by the Ethics Committee of the Institutional Review Board of Gujarat Ayurveda University (IAEC-04/09-10/Proj-02).

2.4.2. Biochemical and hematological analysis

Evaluation of blood biochemistry was carried out in all the animals on Day 15, Day 45, and Day 91 at the time of sacrifice.

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