Chagas disease worldwide are infected by Trypanosoma cruzi, etiologic agent of Chagas disease. The absence of effective trypanocidal chemotherapy reflects the need for constant research in this area. Some compounds such as naphtoquinones can reduce trypanothione reductase (TR), specific enzyme of trypanosomatids, which controls cellular oxidative stress. Inhibition of TR favors an oxidative process and death of the parasite. In this work, we investigate the trypanocidal potential of phytomenadione (K1) and menadione (K3), both vitamins K derived from naphthoquinones. The trypanocidal activity of K1 and K3 was evaluated by in vitro assays with replicative form epimastigote, the bloodstream trypomastigotes and the intracellular form amastigote of Y and Colombian strains. Cytotoxicity was determined by mode of incorporation of [³H]-thymidine. Scanning and transmission electron microscopy were performed to analyze the effect of the treatment with K1 and K3 on the ultrastructural of trypomastigotes. In vivo, trypanocidal activity was evaluated by observing the levels of parasitemia. Our data demonstrated the high trypanocidal activity of K1 and K3. Both compounds were able to inhibit the proliferation of epimastigotes and amastigotes and reduce viability of trypomastigotes in vitro assays. With emphasis of vitamin K3 which showed lower IC₅₀ values, for all the forms, when compared with the reference drug, benznidazole. For example, K3 had an IC₅₀ against trypomastigotes (Y strain) of 2.19 ± 0.02 μM and the positive control benznidazole presented an IC₅₀ of 12.43 ± 0.52 μM. And for amastigotes (Y strain) the IC₅₀ of K3 was 4.90 ± 0.29 μM and the positive control benznidazole was 13.99 ± 0.39 μM. K1 and K3 presented less cytotoxicity on mammalian cells compared to the parasites, demonstrating selective character. Transmission electron microscopy revealed that the treatment with vitamin K1 and K3, with their respectively IC₅₀ values, resulted in kinetoplast and mitochondria disorganization and the appearance of vacuoles. The scanning electron microscopy revealed that the treatment with vitamin K1 and K3 cause the appearance of membrane protusions, shrinkage of the parasites and discontinuities on the surface of the trypomastigotes after treatment. In vivo, the treatment using the dose of 25 mg/kg/day with K3 was able to reduce significantly parasitemia (p < 0.05). New research may propose molecular structural improvements to enhance the activity of K1 and K3, offering thus an effective alternative for chemotherapy against Chagas disease. E-mail: taniramutino@aluno.bahia.fiocruz.br