

Metagenomic and phytochemical analyses of kefir water and its subchronic toxicity study in BALB/c mice

ABSTRACT

In recent years, researchers are interested in the discovery of active compounds from traditional remedies and natural sources, as they reveal higher therapeutic efficacies and improved toxicological profiles. Among the various traditional treatments that have been widely studied and explored for their potential therapeutic benefits, kefir, a fermented beverage, demonstrates a broad spectrum of pharmacological properties, including antioxidant, anti-inflammation, and healing activities. These health-promoting properties of kefir vary among the kefir cultures found at the different part of the world as different media and culture conditions are used for kefir maintenance and fermentation. This study investigated the microbial composition and readily found bioactive compounds in water kefir fermented in Malaysia using 16S rRNA microbiome and UHPLC sequencing approaches. The toxicity effects of the kefir water administration in BALB/c mice were analysed based on the mice survival, body weight index, biochemistry profile, and histopathological changes. The antioxidant activities were evaluated using SOD, FRAP, and NO assays. The 16S rRNA amplicon sequencing revealed the most abundant species found in the water kefir was *Lactobacillus hilgardii* followed by *Lactobacillus harbinensis*, *Acetobacter lovaniensis*, *Lactobacillus satsumensis*, *Acetobacter tropicalis*, *Lactobacillus zae*, and *Oenococcus oeni*. The UHPLC screening showed flavonoid and phenolic acid derivatives as the most important bioactive compounds present in kefir water which has been responsible for its antioxidant activities. Subchronic toxicity study showed no toxicological signs, behavioural changes, or adverse effects by administrating 10 mL/kg/day and 2.5 mL/kg/day kefir water to the mice. Antioxidants assays demonstrated enhanced SOD and FRAP activities and reduced NO level, especially in the brain and kidney samples. This study will help to intensify the knowledge on the water kefir microbial composition, available phytochemicals and its toxicological and antioxidant effects on BALB/c mice since there are very limited studies on the water kefir grain fermented in Malaysia.