



Evaluation of anthelmintic efficacy of ethanolic leaf extract of *Juglans regia* L. on *Ascaridia galli*: a comprehensive in vitro and in vivo study

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Abstract

Anthelmintic resistance in livestock animals has been spreading across the world in prevalence and severity. As a result, researchers are exploring alternative strategies to combat this issue, and one promising avenue is the utilization of medicinal plants. This study aims to investigate the anthelmintic efficacy of the crude ethanolic extract (CEE) derived from the leaves of *Juglans regia* against one of the most detrimental nematode parasites affecting poultry, namely *Ascaridia galli* (*A. galli*). For the in vitro studies, adult *A. galli* worms were collected from the naturally infected chickens and the efficacy of CEE was measured at the concentration of 25, 50, and 100 mg/ml using adult worm motility inhibition (WMI) assay. In addition, levamisole (0.55 mg/ml) was used as the positive control. Likewise, Phosphate buffered saline (PBS) was used as the negative control. For the in vivo studies, CEE of *J. regia* at the doses of 500, 1000, and 2000 mg/kg were evaluated in chickens experimentally infected with *A. galli*. The anthelmintic efficacy was monitored using faecal egg count reduction (FECR) and worm count reduction (WCR) assays. In vitro studies revealed significant ($P < 0.001$) anthelmintic effects of CEE of *J. regia* on the motility of *A. galli* worms at different hours post-exposure. At the concentration of 100 mg/ml, CEE resulted in 96.5% inhibition of worm motility at 24 h post-exposure. While the synthetic anthelmintic drug, levamisole caused the highest inhibition of worm motility (100%) at the same time period. The in vivo anthelmintic activity of CEE of *J. regia* demonstrated a maximum effect on day 14 post-treatment by inducing 67.28% FECR and 65.03% WCR. We observed no significant difference ($P > 0.05$) in worm counts between the negative control group and the chickens treated with CEE at the dosage of 500 mg/kg. Together, the results of the present study suggest that CEE of *J. regia* leaves possess anthelmintic properties and could be a potential source of novel anthelmintic compounds for controlling helminth parasites.

Keywords *Juglans regia* · Anthelmintic resistance · *Ascaridia galli* · Anthelmintic activity · Chicken

Introduction

Over the past few years, free-range poultry production systems have been increasing in many parts of the world. It is generally considered that free-range production systems offer improved welfare for poultry birds. However, these systems also increase the exposure of birds to various gastrointestinal helminth parasites such as *Ascaridia galli* and *Heterakis gallinarum* which can subsequently lead to health problems associated with these parasites, compromising welfare standards (Dao et al. 2019; Feyera et al. 2022). In free-range systems, the birds are in frequent contact with their excreta which ensure completion of nematode life cycles (Wongrak et al. 2015). Among the various helminths, *Ascaridia galli* is an economically important nematode parasite infecting a wide range of birds including chickens

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