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Original Article - Veterinary Medicine

Efficacy of *Laurus nobilis* extract against *Hymenolepis nana* worm *in vitro*

[*Eficácia do extrato de* Laurus nobilis *contra o verme* Hymenolepis nana in vitro]

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ABSTRACT

The worm *Hymenolepis nana* is a prevalent intestinal parasite of *Mus musculus* and *Rattus norvegicus*, distributed throughout. *Hymenolepis nana* infection continues to provide a challenge for contemporary research groups; they should be excluded and monitored in rat populations due to their effects on animal health. *H. nana* worms were isolated from infected mice and divided into 6 groups (n=5) and placed in separate Petri dishes containing PBS, incubated at 37°C. Group 1 served as the untreated control group. Groups 2, 3, 4, and 5 were exposed to *Laurus nobilis* extract at concentrations of 25, 50, 100, and 200 µg/ml, respectively. Group 6 was treated with the reference drug Albendazole at a concentration of 1 ml/ml. After treatment, worm mortality was recorded by noting the time of death at 20, 40, 80, and 120 minutes. Worms were considered dead if they remained motionless for 30 seconds after being gently touched with a surgical needle and the Petri dish was shaken. This was attained (97± 2.5%; 97± 2.5%) after 120 minutes at the 200 µg/ml and albendazole concentrations, respectively. At the same time, the untreated group lasted for long hours without death. The mortality percentage of the *H. nana* worm adults increased by increasing exposure periods. The mortality percentage showed a strong negative correlation with exposure time, with correlation values of R = −0.97 and P = 0.0001 at a 200 µg/ml concentration, and R = −0.80 and P = 0.0001 at a 100 µg/ml concentration. This study demonstrated that herbal remedies have the potential to contribute to the development of new treatments for parasitic diseases, with their derivatives offering opportunities for enhancing drug production and bioactivity.

Keywords: *Hymenolepis nana*, *Laurus nobilis*, worm, Albendazole

RESUMO

O verme Hymenolepis nana *é um parasita intestinal predominante de* Mus musculus *e* Rattus norvegicus, *distribuído por toda parte. Infecção por* Hymenolepis nana *continua a ser um desafio para os grupos de pesquisa contemporâneos; eles devem ser excluídos e monitorados em populações de ratos devido aos seus efeitos na saúde animal. Os vermes* H. nana *foram isolados de camundongos infectados e divididos em 6 grupos (n=5) e colocados em placas de Petri separadas contendo PBS, incubadas a 37°C. O Grupo 1 serviu como grupo de controle não tratado. Os grupos 2, 3, 4 e 5 foram expostos ao extrato de Laurus nobilis em concentrações de 25, 50, 100 e 200 µg/ml, respectivamente. O grupo 6 foi tratado com o medicamento de referência Albendazole em uma concentração de 1ml/ml. Após o tratamento, a mortalidade dos vermes foi registrada observando-se o tempo de morte aos 20, 40, 80 e 120 minutos. Os vermes eram considerados mortos se permanecessem imóveis por 30 segundos após serem tocados gentilmente com uma agulha cirúrgica e a placa de Petri ser sacudida. A taxa de mortalidade foi alcançada (97±2,5%; 97±2,5%) após 120 minutos nas concentrações de 200 µg/ml e albendazol, respectivamente. Ao mesmo tempo, o grupo não tratado permaneceu por longas horas sem morrer. A porcentagem de mortalidade dos adultos do verme* H. nana *aumentou com períodos de exposição mais altos. A porcentagem de mortalidade mostrou uma forte correlação negativa com o tempo de exposição, com valores de correlação de R = -0,97 e P = 0,0001 em uma concentração de 200 µg/ml, e R = -0,80 e P = 0,0001 em uma concentração de 100 µg/ml. Este estudo demonstrou que os remédios fitoterápicos têm o potencial de contribuir para o desenvolvimento de novos tratamentos para doenças parasitárias, com seus derivados oferecendo oportunidades para melhorar a produção e a bioatividade dos medicamentos.*

Palavras-chave: Hymenolepis nana, Laurus nobilis, *verme, Albendazol*

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INTRODUCTION

Intestinal parasitic infections are a major cause of illness and death, particularly in developing countries (Kiani *et al*., 2016). According to the World Health Organization (Report…, 2005), more than 2 billion people globally are persistently infected with at least one gastrointestinal helminth parasite. These infections, classified as 'Neglected Tropical Diseases,' mainly impact the poorest and most disadvantaged communities (Report…, 2005). One such intestinal parasite is *Hymenolepis nana*, a common tapeworm that infects humans (Thompson, 2015). While *H. nana* infection is found globally, it is most prevalent in the temperate regions of developing countries, where poor sanitation and hygiene facilitate its transmission, especially among children (Hamid *et al*., 2015). The infection can significantly impact family units epidemiologically, as the tapeworm can be transmitted directly from person to person and auto-reinfection may occur (Goswami *et al*., 2011). Although *H. nana* infections are usually asymptomatic, severe infections can lead to headaches, weakness, anorexia, abdominal pain, and diarrhea (Sirivichayakul *et al*., 2000). In extreme cases, *H. nana* infections can cause emaciation and diarrhea, which may be life-threatening. The life cycle of the tapeworm can be either direct (where humans acquire the infection by consuming contaminated food and water) or indirect (involving arthropods as intermediate hosts) (Tasawar *et al*., 2004). Immunocompromised individuals can experience auto-infection, with eggs produced, hatched, and completing their life cycle within the intestine of a single host (Hamid *et al*., 2015). Currently, around 80% of the world's pharmaceuticals are derived from natural sources or their derivatives (Li and Vederas, 2009). Therefore, plants are a highly abundant source of bioactive chemical compounds that combat various diseases. Numerous studies have investigated the anthelmintic properties of various plants (Newman and Cragg, 2007). Notably, Semmler et al. have explored the use of plant extracts as remedies for both internal and external parasites due to their biocidal effects (De *et al*., 2010). *Laurus nobilis*, commonly known as "sweet bay" is an evergreen tree or large shrub from the Lauraceae family, native to the Mediterranean region (Oliveira *et al*., 2009). Although numerous studies have investigated the effectiveness of Laurus nobilis in treating diseases and microbial infections, its impact on parasitic infections remains largely unexplored. Moreover, some studies have indicated that *L. nobilis* has antifungal (Caputo *et al*., 2017), antiviral, antibacterial (Simić *et al*., 2004), acaricidal (Mares *et al*., 2023), and insecticidal properties (Fernandez *et al*., 2020). The current study was designed to look into the possible role that extracts of *L. nobilis* leaves might play in anthelmintic activity when tested in vitro.

MATERIALS AND METHODS

The *L. nobilis* extract was prepared from leaves obtained from spice markets in Riyadh, Saudi Arabia. A taxonomist from the Department of Botany at King Saud University verified the plant's botanical identity (No. 24649). The leaves (100g) were air-dried at 40°C, powdered, and then extracted with 70% methanol for 24 hours at 4°C. The extract was concentrated and evaporated using a rotary vacuum evaporator (Yamato RE300, Japan) at 40°C under reduced pressure. The resulting crude extract was stored at -20°C until used in experiments, as outlined by Yang *et al*. (2014), producing a thick, sticky residue. For in vitro experiments, the powdered extract was dissolved in distilled water.

The mice were euthanized and dissected, with the intestines placed in Petri dishes containing 0.9% NaCl to facilitate the release of adult worms from the intestinal mucus. The worms were thoroughly washed with saline multiple times. Each adult worm was then examined under a microscope, and any damaged worms were separated. The remaining intact, living adults were selected for the in vitro study.

Living adult *H. nana* worms, harvested from freshly euthanized mice, were allocated into five groups (n=5) and placed in separate Petri dishes with PBS, incubated at 37°C. Group 1 served as the control, receiving no treatment. Groups 2, 3, and 4 were exposed to *Laurus nobilis* extract at concentrations of 100, 200, and 400mg/mL, respectively. Group 5 was treated with the reference drug Albendazole at 1mg/mL. The activity and survival of the worms were monitored under a microscope. Worm activity was evaluated based on movement and contractions in the neck area. Worms showing no movement when gently stimulated with a soft

brush were transferred to warm PBS (40°C). Complete inactivity upon exposure to the warm solution was considered paralysis. Worm death was confirmed if no physical movement was observed after 30 minutes in the warmer solution (45°C) (Deori and Yadav, 2016). Each experiment was repeated three times for all groups.

A one-way ANOVA was conducted, followed by pairwise comparisons between groups using the Duncan method. Results are presented as means ± standard deviations, with statistical significance set at $p \leq 0.05$. Statistical analysis was carried out using the Origin 2018 software, while means and standard deviations were calculated using Microsoft® Excel 2003.

RESULTS

Exposure of Hymenolepis nana worms to the *Laurus nobilis* extracts produced 100% mortality in the anthelmintic bioassay, especially at 200µg/ml concentration. The mortality % ranged from 14.3-98% after 120 min. The mortality percentage of the *H. nana* adults decreased by increasing exposure periods. The mortality % was highly negatively correlated with exposure times ($y=19.3x+15.5$, $R^2= 0.9356$, $P=.0001$; $y=6.3x-5$, $R^2 = 0.9599$, and $P =.0001$) at 200 and 100µg/ml concentrations, respectively. The overall results of the current study suggest that the extract of *L. nobilis* may possess potential anthelmintic properties, which could potentially be employed in worm management. Phytochemical compounds have biological activity. We conclude that the *L. nobilis* extract has the potential to be useful in managing mice anthelmintic particularly *H. nana*.

The results revealed notable differences in the mortality rates between the 200 µg/ml concentration at 120 minutes and the 100µg/mL concentration at 20 minutes, as compared to the other mortality rates (Table 1). Additionally, significant differences were observed between the control group and the 200µg/mL concentration at both 20 and 40 minutes. The highest mortality was after 40min at 25, 50, and 100µg/mL concentrations. The lowest mortality was observed after 120 min at all concentrations (Figure 1). Significant differences ($P < 0.05$) were observed in the mortality percentage of *H. nana* adults between plant extract concentrations (50, 100, and 200µg/mL) and the 25µg/mL concentration and control group. In general, no significant differences were found among the concentrations, except for the 25 µg/mL concentration (Figure 2).

The mortality ratio of the *Hymenolepis nana* worm increased with increasing exposure periods. The mortality % was highly negatively correlated with exposure times ($y = 19.3x + 15.5$, $R^2 = 0.9599$ and $P =.0001$) at 200 and 100µg/mL concentrations, respectively (Figure. 3; Figure. 4). Whereas the correlation coefficient at 50µg/mL was moderated negatively ($y = 6.3x - 5$, $R^2 = 0.9599$ and $P = 0.0001$), and weak negatively at 25µg/mL concentration ($R = -0.2$ and $P = 0.0001$). The overall results of the current study suggest that the leaf extract of *L. nobilis* may possess potential anthelmintic properties, which could potentially be employed in worm management

Table 1. Means of mortality % (± SE) of *Laurus nobilis* extracts at various exposure periods (20, 40, 80, and 120 minutes) against Hymenolepis nana worm

Concentration µg/mL	Mortality (%)			
	20 min	40 min	80 min	120 min
Control	0.0±0.0c	0±0.0c	0±0.0c	0±0.0c
25	0.0±0.0c	10±4.7abc	13.3±2.5bc	20±1.3c
50	14.3±2.7abc	30±12.5a	50.3±5.5abc	56±2.5bc
100	25.7±3.9abc	33.3±12.4ab	59.7±7.5abc	81±2.5bc
200	41±3.5a	46.3±10.3ab	71.3±7.9abc	97±2.5bc
Albendazole	43±3.5a	49.3±10.3ab	72.3±7.9abc	98±2.5bc

Means with different lowercase letters in rows or columns indicate a significant difference ($p<0.05$), while means sharing the same lowercase letters are not significantly different ($p>0.05$), according to Duncan's Multiple Range Test following ANOVA. All values are presented as mean ± standard error.

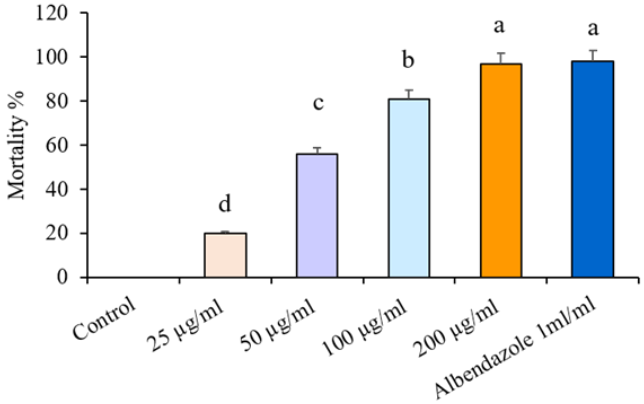


Figure 1. The overall mean (± SE) mortality percentage of *Hymenolepis nana* adults at different concentrations is presented. Values with distinct lowercase letters are significantly different ($p < 0.05$), while those with the same lowercase letters show no significant difference ($p > 0.05$), as determined by Duncan's Multiple Range Test ($p < 0.05$).

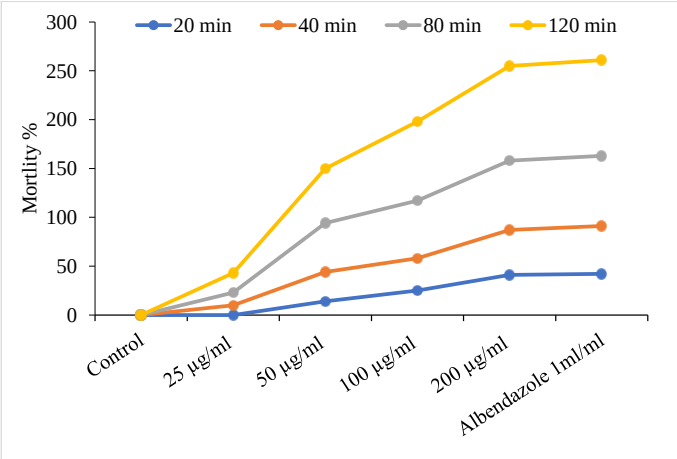


Figure 2. Mortality % of of *Hymenolepis nana* assayed with *Laurus nobilis* extracts at various exposure periods (20, 40, 80, and 120 min).

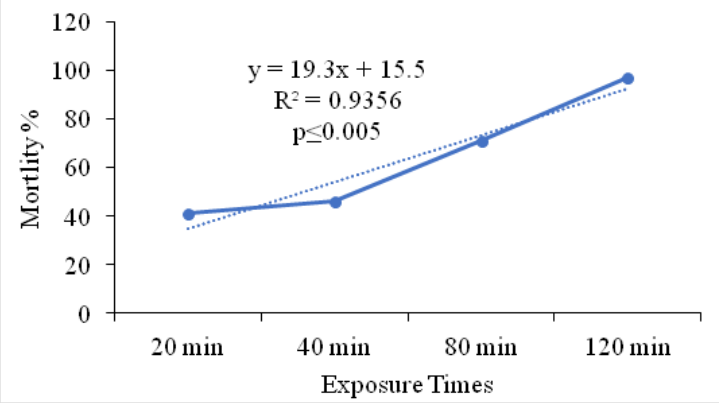


Figure 3. Mean mortality % of *Hymenolepis nana* adults at 200µg/m concentration for various exposure times.

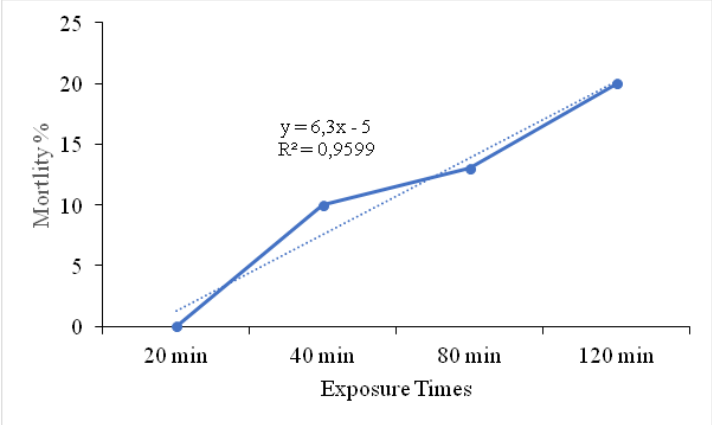


Figure 4. Mean mortality % of H*ymenolepis nana* adults at 25µg/mL concentration for various exposure times (min).

DISCUSSION

Several studies have highlighted the challenges associated with anthelmintic screening and the longevity of drugs (Geary, 2016; Jiao *et al*., 2020). A key limitation in controlling helminthic diseases is the ongoing development of anthelmintic resistance globally (Ploeger and Everts, 2018; Vineer *et al*., 2020). One approach to address this issue is incorporating natural compounds into the management of gastrointestinal parasites. In our study, we observed the mortality rate of adult *H. nana* worms increased as the exposure duration was extended. A strong negative correlation was observed between the mortality rate and the length of exposure time. This study aligns with numerous others that have confirmed the efficacy of *L. nobilis* leaves against a variety of parasites. Sebai *et al*. (2022) demonstrated that the *L. nobilis* extract exhibited significant in vivo anthelmintic activity, eliminating egg production of *Heligmosomoides polygyrus* after 7 days of treatment and reducing total worm counts by 79.2%. The plant's high content of linalool was identified as a key factor in eliminating intestinal worms in infected mice (Sebai *et al*., 2022). In a study conducted by Mares *et al*., 2024 on the Aspiculuris tetraptera parasite, they observed that *L. nobilis* leaves extract had a significant effect on the worms, causing their death both in vitro and in vivo. Furthermore, linalool found in *Cinnamomum camphora* was shown to significantly reduce the number of schistosomulum recovered from mouse skin after infection, leading to a decrease in the worm burden in the infected animals (Batiha *et al*., 2018). As demonstrated by Mares *et al*., 2023 in their study, the methanolic extract of *L. nobilis* leaves can kill *Hyalomma dromedarii* ticks. Fernandez *et al*. (2020) discovered that *L. nobilis* essential oils induced mortality in engorged *Rhipicephalus microplus* females at a concentration of 200µl/mL. Alimi *et al*. (2021) found that the ethanolic extract of *L. nobilis* resulted in 86.2% mortality in engorged female ticks and inhibited egg hatching across all tested concentrations. The leaves of *L. nobilis* have been used as a medicinal herb and exhibit pharmacological properties, including antibacterial and anti-inflammatory effects (Fang *et al*., 2005). In a study the results demonstrated that *L. nobilis* essential oil possesses strong antibacterial effects (Moghtader and Farahmand 2013). The antibacterial activity of *L. nobilis* essential oil was evaluated against several bacterial species, including *E. coli*, *E. faecalis*, and *Salmonella* Pullorum (Tomar *et al*., 2020). Both this study and others have highlighted the effective anti-parasitic properties of *L. nobilis*, suggesting its potential application in parasite control programs.

CONCLUSION

The leaves of *L. nobilis* contain many phenolic compounds that contribute to the antihelmintic properties of this plant. It has also been shown that the *L. nobilis* leaf extract is effective in killing *Hymenolepis nana* worms in vitro. The phenolic compounds are believed to be responsible for these activities. Further research is needed, including more in-depth studies on various types of worms and in vivo studies.

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ETHICAL ASPECTS

The research was submitted to the *Ethics Committee on Animal Use* of the University King Saud University, and approved under the number [Approval No. KSU-Se-21-86]).

CONTRIBUTORS

M. Murshed, M.M. Mares, H. Alzaylaee, S. Al-Quraishy: study concep-tion, objective, data analysis, discussion, manuscript writing, and approval of the version to be published.

DATA AVAILABILITY STATMENT

The research data are available within the article itself.

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