

STUDY OF *Indigofera tinctoria* AS ANTHELMINTIC IN SHEEP
INFECTED WITH *Haemonchus contortus*

ABSTRACT

Hemonchosis has resulted in huge economic losses for sheep farmers. Secondary metabolite compounds from *Indigofera tinctoria* L. can be used as anthelmintics. This study aimed to determine the prevalence of *Haemonchus contortus* on sheep and to evaluate the *in vitro* and *in vivo* effects of *I. tinctoria* L. aqueous extract (IAE) as an anthelmintic against *Haemonchus contortus* in sheep. Prevalence, abomasum samples (200) were collected randomly from the some local slaughterhouse in Sleman Regency, Yogyakarta. Determination of positif animals is based on the discovery of adult worms *H. contortus* in abomasum. *In vitro* test, in the egg hatch test, eggs suspension (1000 eggs) were placed in mikrotiter plat containing 1 ml of IAE at concentrations 100, 120, 140, 160, 180, 200, and 220 mg/mL, albendazole 1%, and 0.62% saline water each experiment was repeated. In the adult worm motility test, ten active adult worms were placed in each Petri dish containing 25 mL of IAE, each having a different concentration of IAE at concentrations 100, 120, 140, 160, 180, 200, and 220 mg/mL albendazole 1%, and 0.62% saline water each experiment was repeated. The ultrastructure of the egg was observed using dengan *Scanning Electrone Microscopy* (SEM) and protein profiles using sodium dodecyl sulfate-polyacrylamide gel electrophoresis. In the *in vivo* study, 15 sheep were used and divided into 5 groups. All groups were infected by 1000 infective larvae per oral every week for 6 weeks. At 9 weeks after infection, group 1 untreated as a control. Groups 2,3,4 were given infusion doses 21 mg/ml, 41 mg/ml dan 62 mg/ml which given per day for 9 days. Group 5 was given albendazole. Clinical examination, hematology test and FECR were conducted every weeks. Sheeps were euthanized for post mortem examination. SEM was performed using worms found from the abomasum of slaughtered sheep. The prevalence of *H. contortus* was observed in sheep (68%). All quantitative data were analyzed statistically, data from SEM, SDS PAGE and histopathology were used for descriptive analysis. The results of *in vitro*, the results of egg hatch test, the 20% dose has greatest inhibitory hatchability (86%). The most effective concentration that killed the adult *H. contortus* worms was 220 mg/mL (93.33% mortality) after 8 h of treatment. The electrophoresis results showed that the protein band at a dose of 22% was less than that of the control. The results of *in vivo*, the average body weight, mean BCS, FAMACHA, Hb, PCV, TEC of post-treatment were increased. The aqueous extract of *I. tinctoria* was decreased FEC. The SEM results showed that IAE treatment caused the worms' anterior parts to become wrinkled with thick creases and cuticle abrasion (*in vitro*) and the anterior part to shrink along with the presence of aggregates in the worm cuticle (*in vivo*). This study showed that the prevalence *H. contortus* in sheep is 68%. The aqueous extract of *I. tinctoria* has an anthelmintic effect with inhibited hatchability, decreased FEC, increased weight gain, BCS, FAMACHA, Hb, PCV, and TEC, and reducing the protein profile of adult *H. contortus* worms.

Keywords: anthelmintic, *Haemonchus contortus*, *Indigofera tinctoria*, scanning electron microscopy, sheep.

INTISARI

Haemonchosis mengakibatkan kerugian ekonomi yang besar terhadap peternak domba. Metabolit sekunder dari *Indigofera tinctoria* dapat digunakan sebagai anthelmintik. Penelitian ini bertujuan mengetahui angka prevalensi *Haemonchus contortus* pada domba, mengetahui pengaruh infusa *I. tinctoria* sebagai anthelmintik terhadap *H. contortus* domba secara *in vitro* dan secara *in vivo*. Penentuan prevalensi, sampel abomasum (200) diambil secara acak dari beberapa tempat pemotongan hewan di Sleman. Penentuan hewan positif didasarkan pada ditemukannya cacing dewasa *H. contortus* di abomasum. Uji *in vitro*, pada uji hambatan penetasan telur, suspensi telur (1000) di tempatkan dalam sumuran plat mikrotiter yang berisi 1 ml infusa *I. tinctoria* dosis 100, 120, 140, 160, 180, 200 dan 220 mg/ml, albendaole 1% dan Nacl fisiologis 0.62% dengan masing-masing 3 ulangan. Uji motilitas cacing dewasa, 10 cacing dewasa aktif di tempatkan dalam cawan petri yang berisi 25 ml infusa *I. tinctoria* dengan dosis 100, 120, 140, 160, 180, 200 dan 220 mg/ml, albendaole 1% dan Nacl fisiologis 0.62% dengan masing-masing 3 ulangan. Uji ultrastruktur permukaan tubuh cacing mati dengan scanning electron microscopy (SEM) dan profil protein dengan sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE). Uji *in vivo*, sebanyak 15 ekor domba di bagi rata menjadi 5 kelompok, semua kelompok diinfeksi 1000 L3 per oral setiap minggu selama 6 minggu. Pada minggu ke 9, kelompok 1 tidak diberi obat. Kelompok 2,3, dan 4 diberikan infusa *I. tinctoria* dengan dosis masing-masing 21 mg/ml, 41 mg/ml dan 62 mg/ml selama 9 hari. Kelompok 5 diberi albendazole 100 mg/ml. Pemeriksaan klinis, hematologi dan EPG dilakukan setiap minggu. Domba di autopsi dan dianalisa patologi. Uji SEM dilakukan pada cacing yang ditemukan pada abomasum. Prevalensi pada domba (68%). Data kuantitatif dianalisis statistik, hasil SEM, SDS-PAGE dan histopatolgi dianalisa deskriptif. Hasil *in vitro*, dosis infusa *I. tinctoria* 220 mg/ml menghambat penetasan paling tinggi (86%). Dosis infusa *I. tinctoria* 220 mg/ml membunuh cacing dewasa 93.33% setelah 8 jam perendaman. Hasil elektroforesis menunjukkan band protein pada dosis 22% lebih sedikit daripada kontrol. Hasil *in vivo*, rata-rata berat badan, BCS, FAMACHA, Hb, PCV dan RBC mengalami kenaikan. Infusa *I. tinctoria* menurunkan nilai FEC. Hasil SEM menunjukkan bahwa infusa *I. tinctoria* menyebabkan bagian anterior cacing menjadi mengkerut dengan lipatan yang tebal dan abrasi kutikula (*in vitro*), dan bagian posterior menyusut dengan adanya agregat pada kutikula (*in vivo*). Penelitian ini menunjukkan bahwa prevalensi *H. contortus* pada domba sebesar 68%, infusa *I. tinctoria* menghambat penetasan telur, menurunkan nilai FEC, meningkatkan berat badan, BCS, FAMACHA, Hb, PCV dan RBC, dan menurunkan profil protein cacing dewasa *H. contortus*.

Kata Kunci: anthelmintik, *Haemonchus contortus*, *Indigofera tinctoria*, domba