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ANTI-TRYPANOSOMA ACTIVITY OF ETHANOLIC EXTRACT OF NEEM LEAF *(Azadirachta indica) ON Trypanosoma evansi IN RATS (Rattus norvegicus)*

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ABSTRACT

The aim of this study was to determine the effect of neem leaf extract *(Azadirachta indica)* on parasitemia of rats infected with *Trypanosoma evansi* (*T. evansi*) Aceh local isolate. A total of 24 male rats aged three months were used in this study and randomly divided into six treatment groups equally. The negative control group (K0) without *T. evansi* infection and neem leaf extract, the positive control group (K1) was infected with *T. evansi* but no neem leaf extract given, group K2, K3, K4, and K5 were infected with $5x10^4$ *T. evansi* and were given neem leaf extract after patent infection with dose of 50, 100, 400, and 800 mg/kg BW respectively. The extract was given orally for three consecutive days. On the fourth day, rat blood was drawn for parasitemia examination. The results showed that no *T. evansi* detected in rats in negative control group (K0), while parasitemia in group K1; K2; K3; K4; and K5 was 12,295 $x10^6$/mL; 10,495 $x10^6$/mL; 9,360 $x10^6$/mL; 5,080$x10^6$/mL; and 2,398$x10^6$/mL of blood, respectively. Percentage of inhibition of parasitemia in K2, K3, K4, and K5 reached 14.64, 23.78, 58.68, and 80.50%, respectively. Based on the result of the study, neem leaf extract of 800 mg/kg BW gave the highest reduction of parasitemia in rats infected with *T. evansi*.

Key words: neem leaf, parasitemia, *T. evansi*

ABSTRAK

Penelitian ini bertujuan mengetahui pengaruh ekstrak daun mimba *(Azadirachta indica)* terhadap parasitemia tikus yang diinfeksikan *Trypanosoma evansi* (*T. evansi*) isolat Aceh. Sebanyak 24 ekor tikus jantan berumur tiga bulan dipakai dalam penelitian ini. Semua tikus dibagi ke dalam enam kelompok perlakuan yang masing masing kelompok terdiri atas empat ekor. Kelompok kontrol (K0) tanpa infeksi *T. evansi* dan ekstrak daun mimba, kelompok perlakuan 1 (K1) diberikan *T. evansi* tanpa ekstrak daun mimba kelompok 2 (K2), kelompok 3 (K3), dan kelompok 4 (K4) berturut turut diberi *T. evansi* dan ekstrak daun mimba dosis 50, 100, 400, dan 800 mg/kg bobot badan. Ekstrak diberi secara oral selama tiga hari berturut-turut. Pada hari keempat, tikus diambil darahnya untuk pemeriksaan parasitemia. Hasil penelitian menunjukkan pada K0 tidak ditemukan adanya *T. evansi*, sedangkan pada K1; K2; K3; dan K4 ditemukan parasitemia masing-masing sebesar $12.295x10^3$/ml; $10.495x10^3$/ml; $9.360x10^3$/ml; $5.080x10^3$/ml; dan $2.398x10^3$/ml. Persentase daya hambat parasitemia pada K2; K3; K4; dan K5 masing-masing adalah 14,64; 23,78; 58,68; dan 80,50%. Hasil penelitian ini menunjukkan bahwa ektrak daun mimba 800 mg/kg paling mempunyai daya hambat pertumbuhan *T. evansi* paling tinggi dibandingkan dosis lainnya.

Kata kunci: daun mimba, parasitemia, T. evansi

INTRODUCTION

Animal trypanosomiasis (surra) is a deadly, fly-born parasitic disease for some animal species caused by the blood protozoan *Trypanosoma evansi* (*T. evansi*) (Luckins *et al.*, 1992). The parasite that has the widest host range and geographical distribution is transmitted by the bite of infected horse flies (*Tabanus* spp.) and stable flies (*Stomoxys* spp.) (Bawm, 2010). The host includes wildlife such as deer, elephant, rhinoceros, and tapir; and domestically high economic value animals such as cattle, horse, and buffalo (Vellayan *et al*., 2004; Adrian *et al*., 2010). This disease has become a major obstacle to livestock industry and economic developments and thus become important priority for biomedical and public agencies, agricultural sector and the scientist in some countries (WHO, 2001; Aksoy, 2003).

Clinical manifestation of surra in animals are varied greatly depending upon isolates of the parasite and animal species infected (Luckins, 1996; Desquesnes *et al*., 2013). As consequences the disease is not only multispecies but also polymorphic (Desquesnes *et al.*, 2013).

Chemotherapy and chemoprophylaxis are still the main way in controling animal trypanosomiasis. Currently, there are six coumpounds available for treatmen of animal trypanosomiasis (diminazene azeturate, isometamedium chloride, homidium bromide/ homidium chloride, quinapyramine sulphate/sulphate chloride, suramine sodium, and melarsomine dihydrochloride). However, there are increasing evidences that drug resistance were observed in some countries (Giordani *et al.*, 2016). For instance, Tsegaye *et al.* (2015) reported that resistance of trypanosome to anti-trypanosomal drugs occurs in 21 African countries and multiple resistances occur in 10 African countries. Resistance of trypanosomes to anti-trypanosomal drugs was also reported in other countries. In China, resistance of some isolates of *T. evansi* was observed to Suramin and Antrycide (Zhou *et al.*, 2004). In Indonesia, some *T. evansi* isolates are resistance to isometamedium and some are resistance to diminaze azeturate (Sukanto *et al.*, 1987). These condition sparked research toward new trypanosomacide to anticipate total resistance.

Last few decades, research toward new drug has been focused on plants based drugs and neem (*Azadirachta indica*, *A. indica*) is one of herb that has been extensively explored for its potency (Kardiman and Dhalimi, 2003). Previous studies have shown that neem has antimalarial activity (Setiawan, 2009; Isa *et al*., 2012). *Trypanosoma evansi* and plasmodium are both blood parasite that share the same class in taxonomy, therefore, it is assumed that neem has antitrypanosoma activity. In this article, we describe the potency of ethanol extracted neem leaves in reducing parasitemia level in rats.

MATERIALS AND METHODS

Trypanosoma evansi

Trypanosoma evansi used in this study was a local isolate obtain from whole blood of buffalo in Aceh Besar, Aceh Province, Indonesia. The isolate has been cryopreserved in liquid nitrogen.

Neem (*Azadirachta indica*)

Sufficient numbers of neem leaves were taken from the field and air dried. The leaves were macerated and ethanol extracted. Maceration was done 3 x 24 hours and the solution was filtrated. The filtrate was then evaporated to eliminate solvent (BPOM, 2000).

Experimental Design

This study used a completely randomize design with six treatment groups and four replications each. Here, 24 male rats (*Rattus norvegicus*) aged three months old were equally assigned into six groups as the following: K0 was untreated rats (negative control) whereas K1 (positive control), K2, K3, K4, and K5 were rats infected with $5x10^4$ *T. evansi* and treated with 0, 50, 100, 400, and 800 mg/kg BW neem leaves extracts, respectively.

Anti-trypanosomal Test

Trypanosoma evansi isolate was thawed at room temperature, diluted in 100 µl of phosphate buffer saline glucose (PBSG), and intraperitoneally injected into a rat. Parasitemia was checked every two days using improved Neubauer counting chamber for leucocyte. When parasitemia reached 10^7-10^8/mL, blood samples were collected and used for the infection step. Here, rats were individually injected with $5x10^4$ *T. evansi* and given neem leaves extracts orally for three consecutive days after patent infection. On day 4, blood samples were collected and the numbers of parasites were microscopically determined using improved Neubauer chamber for leukocyte and counted using the formula below.

Numer of parasite/mL = A x B x 10^4

A = number of parasites counted
B = dilution factor

RESULTS AND DISCUSSION

Levels of parasitemia in *T. evansi* infected rats treated with different doses of *A. indica* leaf extracts are presented in Table 1. While rats from negative (un-infected) control showed no parasitemia, infected rats given ethanolic extract of the medicinal plant of 0 mg/kg BW (K1), 50 mg/kg BW (K2), 100 mg/kg BW (K3), 400 mg/kg BW (K4) and 800 mg/kg BW (K5) had parasitemia $12.30x10^3$/mL, $10.5x10^3$/mL, $9.4x10^3$/mL, $5.1x10^3$/mL, and $2.4x10^3$/mL, respectively.

Table 1. Parasitemia and average reduction of parasitemia in *T. evansi* infected rats treated with various doses of neem leaves extract for three consecutive days

Treatment group	Parasitemia ($x10^6$/mL)	Relative inhibition (%)
K0, negative control (no infection and no neem leaves extract given)	0.00	-
K1, positive control (infected with *T. evansi* and no neem leaves extract given)	12.29	-
K2, infected with *T. evansi* and given 50 mg/kg BW neem leaves extract	10.49	14.64
K3, infected with *T. evansi* and given 100 mg/kg BW neem leaves extract	9.36	23.87
K4, infected with *T. evansi* and given 400 mg/kg BW neem leaves extract	5.08	58.68
K5, infected with *T. evansi* and given 800 mg/kg BW neem leaves extract	2.40	80.50

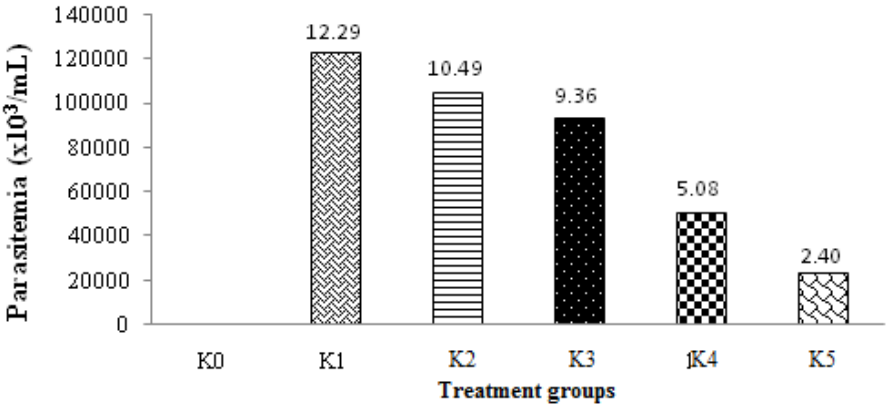

Figure 1. Parasitemia from *Trypanosoma evansi* infected rats treated with various doses of neem leaves extract

Parasitemia suppression caused by the respective dose of the extracts on the growth of *T. evansi in vivo* was 14.6, 23.9, 56.7, and 80.5%, respectively.

Reduced parasitemia in *T. evansi* infected rats given increasing doses of ethanolic extracts of *A. indica* (Figure 1) indicated a dose-dependent anti-trypanosomal activity of the extract. This finding was in agreement with result reported by Raphael *et al*. (2009) when evaluating chemo-preventive effect of methanolic extracts of *A. indica* on *T. brucei* in dogs. When given at higher doses, the extract even showed comparable to or better efficacy than the well-known commercially available trypanocidal drug suramin.

Anti-trypanosomal potential of *A. indica* plant is supported by better bioavaiability and lower toxicity of the whole extract (Aggarwal *et al*., 2011). In contrast, lower parasitemia level of *T. evansi* of rats administrated with higher doses of *A. indica* showed toxicity effect of the extract on the parasite. The toxicity was one of mechanisms responsible for better antitrypanosoma effects of some medicinal plants against *T. cruzi* (Teixeira *et al*., 2014).

The ability of *A. indica* extract to reduce or kill trypanosome parasites and other apicomplexan blood protozoan such as plasmodium (Momoh *et al*., 2015) and Leishmania (Teixeira *et al*., 2014) can be attributed to high phytochemical contents of the extract. Methanolic extract of *A. indica* contains some secondary metabolites like tannins, saponins, flavonoids, and glycoside (Momoh *et al*., 2015). The occurrences of these secondary metabolites were identified by other researchers who found alkaloid, terpenoid, kuinolid, phenolic, flavonoid substances in the neem plant extract (Karira *et al*., 2004; Puspitasari *et al*., 2009). The neem leaves extract also contains tannin and saponin (Puspitasari *et al*., 2009). Tasdemir *et al*. (2006) added that flavonoid had activity as growth inhibitor for *T. brucei* at trypomastigote stage. Ekaningtias (2014) supported that terpenoid compound is able to inhibit ATP production that is crucial in development of *T. evansi.*

These compounds might be available in other parts of the plants since all parts of the *A. indica* plant are useful and have been used in treatment of diseases ranging from tooth decay, swollen liver, ulcers, dysentery, diarrhea, malaria, and other bacterial infections (Allameh *et al*., 2002; Mossini *et al*., 2004).

CONCLUSIONS

Rat that survived from *T. evansi* infection after administration of various doses of ethanol extracted neem leaves proved that the neem leaves have anti-trypanosomal activity. It was also proven that the higher the dose the higher percentage of anti-trypanosomal activity.

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