NOTE *Internal Medicine*

Effects of Central Kalimantan Plant Extracts on Intraerythrocytic *Babesia gibsoni* in Culture

SUBEKI[1)], Hideyuki MATSUURA[1)], Masahiro YAMASAKI[2)], Osamu YAMATO[2)], Yoshimitsu MAEDE[2)], Ken KATAKURA[3)], Mamoru SUZUKI[3)], TRIMURNINGSIH[4)], CHAIRUL[4)] and Teruhiko YOSHIHARA[1)]*

[1)]Laboratory of Bioorganic Chemistry, Division of Applied Bioscience, Graduate School of Agriculture, Hokkaido University, Sapporo 060–8589, [2)]Laboratory of Internal Medicine, Department of Veterinary Clinical Sciences, Graduate School of Veterinary Medicine, Hokkaido University, Sapporo 060–0818, [3)]Department of Parasitology, Gunma University School of Medicine, Maebashi 371–8511, Japan and [4)]Department of Research and Development for Biology, Indonesian Institute of Sciences, Bogor 16122, Indonesia

(Received 17 September 2003/Accepted 7 February 2004)

ABSTRACT. The inhibitory effects of 45 plant extracts selected from Central Kalimantan, Indonesia on *Babesia gibsoni in vitro* and their acute toxicity to mice were evaluated. Of these plant extracts studied, *Arcangelisia flava, Curcuma zedoaria, Garcinia benthamiana, Lansium domesticum* and *Peronema canescens* were found to have appreciable antibabesial activity with IC_{50} values from 5.3 to 49.3 μg/m*l* without acute toxicity in mice at the intraperitoneal dose of 0.7 g/kg of body weight.
KEY WORDS: antibabesial activity, *Babesia gibsoni*, Central Kalimantan plant, toxicity.

J. Vet. Med. Sci. 66(7): 871–874, 2004

Canine babesiosis is a tick-borne disease of wild and domestic dogs caused by intraerythrocytic parasites, *Babesia gibsoni* and *Babesia canis*. The parasites are widely distributed in tropical and subtropical areas worldwide and cause hemolytic anemia of the affected dogs [2, 5, 22]. Diminazene aceturate (Ganazeg) is an aromatic diamidine used extensively for the treatment of *B. gibsoni* infection in affected areas of the world [5]. Although the medicine can reduce the severity of clinical signs and the mortality associated with the disease, it sometimes induces severe side effects such as weakness, irritability, paralysis, non-responsiveness to stimuli and fatal central nervous system haemorrhage [3]. Dogs that have recovered from the babesiosis also commonly become chronic carriers, thereby posing a source of infection for other dogs and ticks. Notably, production of this drug was recently stopped. Therefore, for the treatment of *B. gibsoni* infected dogs, an alternative chemotherapeutic agent having few side effects is urgently needed. One possible source of such affordable treatment lies in the use of plant extracts.

Central Kalimantan, Indonesia is well known for its green tropical vegetation and peat swamp forests. Its diverse nature and uses are claimed to have medicinal properties. No data are available to assess the extent to which these plant extracts can be used for the treatment of *B. gibsoni* infected dogs. The aims of the present study were to evaluate the inhibitory effects of the Central Kalimantan plant extracts on the *B. gibsoni in vitro* and their acute toxicity to mice.

Forty-five plants were collected from Central Kalimantan, Indonesia based on ethnopharmacological characteristics. These plants were identified at the Herbarium Bogoriense, Indonesia. Voucher specimens were deposited at the Department of Research and Development for Biology, Indonesian Institute of Sciences, Bogor. Each plant sample (10 g) was boiled for 30 min in 200 m*l* of water twice. The boiling water was freeze-dried and then ground into powder.

To evaluate the antibabesial activity, the powder of plant extracts was dissolved in dimethyl sulfoxide (DMSO) and further diluted with RPMI 1640 medium supplemented with sodium pyruvate (0.1 mg/m*l*), glutamine (0.3 mg/m*l*), sodium bicarbonate (2 mg/m*l*), penicillin (100 units/m*l*) and streptomycin (100 μg/m*l*). The final DMSO concentration was not more than 0.1%. *B. gibsoni* used in this study was maintained in the culture medium according to a modified method of Murase *et al.* [12]. Heparinized venous blood from a normal dog was washed three times with Vega y Martinez (VYM) solution [21], and then washed twice with RPMI 1640. After the washing, erythrocytes were resuspended to a final packed cell volume of 6% in a culture medium consisting of 60% RPMI 1640 and 40% normal dog serum. The erythrocyte suspension was mixed with parasitized erythrocytes to get 0.5–1.0% parasitemia at the start of incubation. The test was done in a 96-well culture plate and each well contained 25 μl of parasitized erythrocyte suspension and 25 μl of extract solution containing the respective concentrations of each extract. Final concentrations of the extracts were 0, 1.9, 3.9, 7.8, 15.6, 31.2, 62.5, 125, 250, 500 and 1,000 μg/m*l*. The plate was incubated at 37°C under a humidified atmosphere of 5% CO_2, 5% O_2 and 90% N_2. After keeping the plate in an incubator for 72 hr, a Giemsa stained thin smeared specimen was prepared and the parasitemia level was determined by counting the number of parasitized cells in 1,000 erythrocytes.

Plant extracts having activity were then tested for acute toxicity to mice at a dose of 0.7 g/kg of body weight. The administered dose (0.7 g/kg) was determined according to a

* CORRESPONDENCE TO: YOSHIHARA, T., Laboratory of Bioorganic Chemistry, Division of Applied Bioscience, Graduate School of Agriculture, Hokkaido University, Sapporo 060–8589, Japan.

lethal dose (LD_{50}) of antimalarial plant extract of *Enantia chlorantha* [1]. Five mice (SLc: ICR, male, body weight 28 ± 2 g) per group were housed in metal cages and given a standard laboratory diet and water *ad libitum*. Each mice was intraperitoneally given extract on the first day of the experiment. Each dose was given in 0.2 m*l* of 0.1% carboxymethyl cellulose (CMC) solution. One group, which was given CMC solution, served as the control. The toxicity was determined by observing which mice were dead or alive for 7 days after administration.

Antibabesial activities of the 45 plant extracts on *B. gibsoni in vitro* are shown in Table 1. Twenty-one extracts, *A. flava, A. scholaris, C. zedoaria, C. glaucum, D. fruticosum, E. petiolatus, E. parvifolius, E. longifolia, G. benthamiana, G. rigida, L. domesticum, M. paniculatus, N. lappaceum, P. motleyi, P. canescens, P. javanica, P. niruri, P. suaveolens, Q. indica, S. emarginatum* and *S. pycnanthum*, had evident antibabesial activity with IC_{50} values ≤ 62.5 μg/m*l*, while the remaining extracts were less active with IC_{50} values > 62.5 μg/m*l*.

Of the 21 extracts selected, *A. flava, C. zedoaria, G. benthamiana, L. domesticum* and *P. canescens* were found to have appreciable antibabesial activity with IC_{50} values from 5.3 to 49.3 μg/m*l*, and no mortalities in mice at the

Table 1. Antibabesial activity of Central Kalimantan plant extracts against *Babesia gibsoni in vitro*

Scientific name	Family	Part used	IC_{50} (μg/m*l*)
Asclepias curassavica	Asclepiadaceae	leaves	777.8 ± 26.3
Artocarpus heterophyllus	Moraceae	leaves	193.5 ± 14.5
Alstonia scholaris	Apocynaceae	leaves	61.2 ± 1.8
Annona reticulata	Annonaceae	leaves	212.3 ± 9.2
Arcangelisia flava	Menispermaceae	twig	5.3 ± 0.3
Aromadendron nutans	Magnoliaceae	bark	125.4 ± 4.3
Cananga odorata	Annonaceae	leaves	199.9 ± 9.6
Cassia siamea	Fabaceae	leaves	116.7 ± 6.3
Cassia fistula	Fabaceae	leaves	182.2 ± 9.9
Cassia alata	Fabaceae	leaves	403.4 ± 12.3
Carica papaya	Caricaceae	leaves	274.7 ± 7.2
Cerbera manghas	Apocynaceae	leaves	557.6 ± 36.8
Curcuma zedoaria	Zingiberaceae	rhizome	41.7 ± 1.3
Cratoxylum glaucum	Guttiferae	bark	49.8 ± 2.4
Decaspermum fruticosum	Myrtaceae	leaves	46.0 ± 3.2
Elaeocarpus petiolatus	Elaeocarpaceae	bark	4.8 ± 0.4
Elaeocarpus parvifolius	Elaeocarpaceae	bark	12.6 ± 0.7
Elephantopus scaber	Asteraceae	whole	476.1 ± 33.9
Eurycoma longifolia	Simaroubaceae	root	8.4 ± 0.7
Fibraurea chloroleuca	Menispermaceae	leaves	336.8 ± 4.1
Garcinia rigida	Guttiferae	bark	59.3 ± 3.8
Garcinia benthamiana	Clusiaceae	bark	16.3 ± 1.2
Imperata cylindrica	Poaceae	rhizome	197.6 ± 2.9
Lansium domesticum	Meliaceae	bark	49.3 ± 1.5
Mallotus paniculatus	Euphorbiaceae	leaves	47.1 ± 1.7
Melastoma malabathricum	Melastomataceae	leaves	101.9 ± 5.8
Morus alba	Moraceae	leaves	225.8 ± 25.7
Michellia champaca	Magnoliaceae	leaves	686.2 ± 4.4
Microcos lanceolata	Tiliaceae	bark	99.7 ± 1.2
Nauclea subdita	Rubiaceae	root	171.5 ± 14.2
Nephelium lappaceum	Sapindaceae	bark	35.6 ± 0.9
Pentaspadon motleyi	Anacardiaceae	leaves	5.6 ± 0.3
Peronema canescens	Verbenaceae	leaves	43.8 ± 3.5
Picrasma javanica	Simaroubaceae	leaves	18.8 ± 1.5
Pleiocarpidia enneandra	Rubiaceae	root	89.4 ± 5.5
Pongamia pinnata	Fabaceae	leaves	284.3 ± 10.7
Pangium edule	Flacourtiaceae	leaves	197.2 ± 8.6
Poikilospermum suaveolens	Cecropiaceae	leaves	56.0 ± 3.5
Phyllanthus niruri	Euphorbiaceae	whole	9.6 ± 0.3
Quassia indica	Simaroubaceae	leaves	10.5 ± 0.6
Sandoricum emarginatum	Meliaceae	bark	17.4 ± 0.8
Syzygium pycnanthum	Myrtaceae	bark	9.7 ± 0.6
Tetracera scandens	Dilleniaceae	root	86.1 ± 3.0
Tinospora tuberculata	Menispermaceae	stem	390.2 ± 10.2
Timonius billitonensis	Rubiaceae	bark	78.1 ± 1.7

Values are the mean ± standard deviation of three experiments.

Table 2. Toxicity of 21 selected extracts of Central Kalimantan plants to mice

Scientific name	Part used	*N*	Number of death	Day of death	Percent of death
Arcangelisia flava	twig	5	0	0	0
Alstonia scholaris	leaves	5	5	1st	100
Curcuma zedoaria	rhizome	5	0	0	0
Cratoxylum glaucum	bark	5	4	1st-2nd	80
Decaspermum fruticosum	leaves	5	5	2nd	100
Elaeocarpus petiolatus	bark	5	5	1st	100
Elaeocarpus parvifolius	bark	5	5	1st	100
Eurycoma longifolia	root	5	5	1st	100
Garcinia benthamiana	bark	5	0	0	0
Garcinia rigida	bark	5	5	1st	100
Lansium domesticum	bark	5	0	0	0
Mallotus paniculatus	leaves	5	4	1st-2nd	80
Nephelium lappaceum	bark	5	5	1st	100
Pentaspadon motleyi	leaves	5	5	2nd	100
Peronema canescens	leaves	5	0	0	0
Picrasma javanica	leaves	5	5	1st	100
Phyllanthus niruri	whole	5	5	2nd	100
Poikilospermum suaveolens	leaves	5	3	2nd	60
Quassia indica	leaves	5	5	1st-2nd	100
Sandoricum emarginatum	bark	5	5	2nd	100
Syzygium pycnanthum	bark	5	5	2nd-3rd	100

N number of mice per group was intraperitoneally injected with plant extract at a dose of 0.7 g/kg of body weight.

intraperitoneal dose of 0.7 g/kg of body weight (Table 2). The other extracts proved acutely toxic and may be harmful in the treatment of *B. gibsoni* infected dogs. The acute toxic effects in mice might be due to the presence of toxicological constituents in the plant extracts.

A. flava is an important component of folk medicine for the treatment of jaundice, stomachic, anthelmintic, smallpox and aphtha [13]. The phytochemical investigation of *A. flava* has previously shown the presence of berberine, hydroxyberberine, columbamine, jatrorhizine, palmatine, thalifendine, dehydrocorydalmine, shobakunine, limacine, homoaromaline, pycnarrhine and furanoditerpenes [4, 10]. In the present study, extracts of *A. flava* twig displayed very high antibabesial activity with IC_{50} 5.3 μg/m*l*.

C. zedoaria rhizome is widely used as stimulant, stomachic, carminative, diuretic, antidiarrhoeal, antiemetic, antipyretic and depurative [13]. Sesquiterpenes, aerugidiol, curdione, curcumin, curcumanolide, curcumenone, curcumenol, furanodiene, germacrone, isocurcumenol, neocurdione, zedoarol and zedoarondiol have been isolated from this plant and some of them showed an inhibitory effect on D-galactosamine and lipopolysacharide-induced acute liver injury in mice [9, 11, 18, 19]. The rhizome extract of this plant displayed moderate antibabesial activity with IC_{50} 41.7 μg/m*l*.

Genus *Garcinia* comprises about 200 species of polygamous trees to shrubs, mainly distributed in South-East Asian regions [20]. Various species such as *G. indica, G. mangostana* and *G. kola* have been extensively studied as anti-human immunodeficiency virus, antimicrobial, antihepatoxic, antioxidant, antiinflammatory and antiulcerogenic [7, 17]. *Garcinia* is well known for the presence of xanthones, flavonoids, benzophenones, lactone and phenolic acids [20]. Extract of the *G. benthamiana* bark showed high antibabesial activity with IC_{50} 16.3 μg/m*l*.

L. domesticum is a plant native to the Indomalaysia regions widely cultivated for its edible fruit. Bark of this plant can be used for the treatment of dysentery, malaria and as an antidote of scorpion poisoning [13]. Lansic acid, lansiolic acid, dukunolide and cycloartanoid triterpene have been previously isolated from this plant and some of them showed an inhibitory effect on skin tumor promotion [6, 14–16]. The aqueous leaf extracts of this plant were also found to reduce *Plasmodium falciparum* growth in culture [23]. Extract of the *L. domesticum* bark showed moderate antibabesial activity with IC_{50} 49.3 μg/m*l*.

P. canescens is used as a commercial source of timber in South-East Asia regions [20]. Leaves of the plant are traditionally used for the treatment of toothache, fever and dermatosis [13]. Clerodane diterpenoids, named peronemins A2, A3, B1, B2, B3, C1 and D1, have been isolated from the *P. canescens* leaves as an antimalarial agent [8]. The leaf extract of this plant showed moderate antibabesial activity with IC_{50} 43.8 μg/m*l*.

The results given in this paper are a preliminary evaluation of the most interesting plant species and provide additional biological data on Central Kalimantan plants. It would be of interest to further investigate, isolate and identify the possible active principle components of the extracts towards development of antibabesial drugs.

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