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Activity and Toxicity of *Tinospora bakis* in Rats

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Abstract: The activity of *Tinospora bakis* was evaluated *in vivo* against *Trypanosoma evansi* infection in rats. The plant extracts were administered orally at dose rates of 100, 250 and 500 mg kg⁻¹ b.wt. for both methanolic and chloroformic extracts. The result was compared to Cymelarsan which was given at the recommended dose of 2.5 mg kg⁻¹, b.wt. subcutaneously. Daily programs for the parasitaemia for all methanolic and chloroformic extracts in infected or uninfected rats were followed for 30 days after treatment. Blood was collected every week for analysis. *Tinospora bakis* extract was found to be effective in cleaning the parasite for a considerable time. The plant was found to be toxic only with the high dose (500 mg kg⁻¹ b.wt.) of the chloroformic extract. There was an increase in the level of GOT, GPT, Alkaline phosphatase and to some extent in the urea and creatinine. There were necrotic foci in the liver of the rats treated with the high dose of the chloroformic extract.

Key words: *Tinospora bakis*, *Trypanosoma evansi*, toxicity

INTRODUCTION

Trypanosomosis is a group of diseases caused by flagellated protozoan parasites of the genus *Trypanosoma*, family Trypanosomatidae. They are widely distributed in Africa, South America, Asia and the Middle East (Molyneux and Ashford, 1983). *Trypanosoma evansi* causes a disease referred to as Surra. It's an important disease of livestock in Africa and Asia causing great economic losses in camels and water buffalos. *Trypanosoma evansi* is mechanically transmitted during feeding of blood sucking Diptera especially Tabanid flies, (Leach and Roberts, 1981). Since 1961 no additional drugs for use against animal trypanosomosis have gone beyond the experimental stage. Drug resistance between diamidines and isometamidium group seems to exist (El-Rayah *et al.*, 1999). The situation has gotten worse by the slow development of new trypanocidal drugs. An ethnobotanical approach in collaboration with traditional healers remedies may prove to be a rich source of drug discovery (Farnsworth *et al.*, 1985). Herbal medicine is a common practice all over the world. Elhardallou (2011) studied the cytotoxicity and biological activity of many Sudanese medicinal plants. At the recommended dose Cymelarsan® has been shown to be well tolerated (Biswas and Hunter, 1993). Aqueous and methanolic extracts of *Tinospora bakis* were tested for the treatment of malaria caused by *Plasmodium falciparum* and found

to be effective (Ouattara *et al.*, 2006). In Sudan it is used by traditional healers for the treatment of sleeping sickness.

MATERIALS AND METHODS

***Tinospora bakis*:** Was collected from the Angasana hills in Eastern South of Sudan.

Description: Stem terete, scarcely lenticellate and often producing filiform aerial roots. Young stems green with a smooth surface; older ones have a warty surface due to the presence of circular lenticels. Fracture is fibrous. Taste intensely bitter and odorless.

Animals: Swiss albino rats (albino Wistar) were obtained from the laboratory of experimental animals, unit of the veterinary research institute, Soba Khartoum Sudan. They were housed in laboratory cages, fed with pellets and fresh vegetables and were watered *ad libitum* throughout the experimental period.

***Trypanosoma evansi*:** The parasite was isolated from naturally infected camels at Abuzeid livestock market, Omdurman Town. Infected blood was inoculated into a mouse for propagation and at peak of parasitaemia, the infected mouse blood was preserved in liquid nitrogen.

Trypanosomes were counted according to the rapid matching wet-count technique described by Herbert and Lumsden (1976).

Experimental design: Eight groups of ten rats each aged 4-6 weeks average weights 150 g were used. They were divided into 8 groups as follows:- Group 1; infected untreated control. The mice were inoculated with *T. evansi* infected blood at a dose of 5×10^5 intraperitoneally. Group 2; infected and treated with Cymelarsan at a dose rate of 0.25 mg kg^{-1} , b.wt. Group 3, 4 and 5 were infected then treated with methanolic extracts of *T. bakis* at various doses of 500, 250 and 1000 mg kg^{-1} b.wt., respectively. Groups 6, 7 and 8 were infected then treated with chloroformic extracts of *T. bakis* at doses of 500, 250 and 1000 mg kg^{-1} , b.wt., respectively.

Biochemical tests: Serum Glutamic Pyruvic Transaminases (GPT) and serum Glutamic Oxaloacetic Transaminase (GOT), were measured according to the methods of Reitman and Frankel (1957) and Schmidt and Schmidt (1963). Activity of alkaline phosphatase was determined by an optimized standard method according to the recommendation of the Deutsche Gesellschaft für Klinische Chemie. Total protein is determined according to the methods described by Dumas (1972) and Walsh (1983).

Statistical analysis: When three or more observations were made, the results were expressed as Means±Standard Error (SE). The significance of difference between the means were analyzed by the Student t-test (Snedicor and Cochran, 1967).

Histopathological examination: Liver of the sacrificed mouse were taken and immersed in 10% formalin solution, the specimens were trimmed, washed and dehydrated in ascending grades of alcohol. Then cleared in xylol, embedded in paraffin, sectioned (4-6 microns) and stained with hematoxylin and Eosin for histopathological examination to the method of Zimmermann *et al.* (1979).

RESULTS

Cymelarsan was used as a standard drug in this experiment at a dose rate of 0.25 mg kg^{-1} , b.wt. It was clear that there was an immediate cure from the second day of treatment. All the rats became aparasitaemic till the end of the treatment period with percentage clearance 100% (Table 1). The best effect of the extract was given with the high dose (500 mg kg^{-1} , b.wt.) either with the methanolic or chloroformic extract. The initial trypanosome clearance occurred on day 8 with chloroformic extract and on day 10 with methanolic extract with a percentage clearance of 50%. Relapse occurred between the 10th and 15th days of treatment. Biochemical changes revealed increase in the level of GOT, GPT, alkaline phosphatase and to some extend in the urea and creatinine (Table 2). Histopathological finding showed necrotic foci in the liver (Fig. 1).

DISCUSSION

The search for an active trypanocidal drug from a plant origin is a concern of many researchers. For *Tinospora bakis* only the high dose for both methanolic and chloroformic extract appeared to clear the parasite

Table 1: Antitrypanosomal activity of *Tinospora bakis* extracts compared to cymelarsan

Treatment	Group No.	Dose used (mg kg^{-1} b.wt.)	Initial trypanosomes clearance (day)	Relapse	Time to death (day)	Percentage
Infected (untreated control)	1		None		2-12	
Treated with Cymelarsan	2	0.25	2		Dissected on 25	100
Treated with methanolic extract	3	500	10th	Between 10-15	Between 12-24	50
	4	250	14th	Between 7-12	Between 12-20	
	5	100	—	—	Between 9-15	
Treated with chloroformic extract	6	500	8th	Between 10-15	Between 12-20	50
	7	250	12th	Between 9-14	Between 10-16	
	8	100	—	—	Between 10-15	

Each group was composed of 10 rats each. The parasite was given at a dose rate of 5×10^5

Table 2: Biochemical changes in mice associated with treatment by the chloroformic extract of *T. bakis* (500 mg kg^{-1} , b.wt.)

Parameter	Week 0	Week 1	Week 2	Week 3
GOT (U L^{-1})	12.38±1.30 ^{ns}	15.81±0.25 ^{ns}	18.25±0.83 ^{ns}	30.50±0.68*
GPT (U L^{-1})	17.25±0.77 ^{ns}	22.25±0.87 ^{ns}	29.00±0.62 ^{ns}	34.00±0.66*
Total protein (g dL ⁻¹)	6.79±0.49 ^{ns}	6.21±0.59 ^{ns}	5.17±0.49 ^{ns}	4.25±0.62 ^{ns}
Alkaline phosphatase (U L^{-1})	1955±9.55 ^{ns}	2084.01±40.89 ^{ns}	2106.80±18.13 ^{ns}	2314.01±40.63*
Urea (mM L^{-1})	4.23±0.52 ^{ns}	5.92±0.63 ^{ns}	7.03±0.36 ^{ns}	9.21±0.42*
Creatinine (mM L^{-1})	0.51±0.13 ^{ns}	0.61±0.19 ^{ns}	0.84±0.09 ^{ns}	1.04±0.11*

GOT: Glutamic oxaloacetic transaminase, GPT: Glutamic pyruvic transaminase, Values were expressed as Mean±SD, ns: Not significance, * Significant

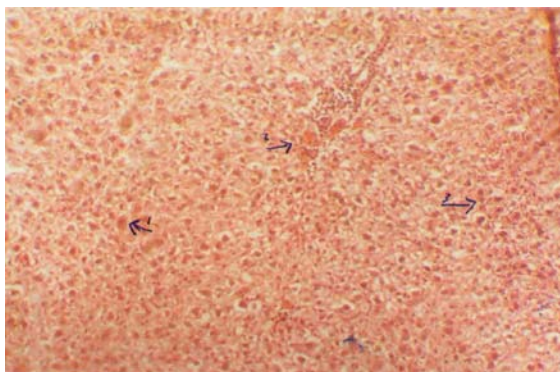


Fig. 1: Necrotic foci in the liver of rat treated with chloroformic extract, 500 mg kg⁻¹ b.wt.

although clearance was only 50%. The lower doses of the plant caused general decrease in the mean parasitaemia count without clearance from blood. This suggested that plant extract might clear the parasite from the blood if the concentration of the plant was increased. Plant extracts were always produced from dried plant material, which may not be the products used traditionally and so disintegration of unstable bioactive compounds might have occurred. In many cases, plants are used in combination with others, which may give rise to synergistic effects. The result of *T. bakis* was found to be similar to that obtained by De-Mesquita *et al.* (2005) who stated that *T. bakis* has a trypanocidal activity against *T. cruzi*. This result also found to resemble the results obtained by Ouattara *et al.* (2006) who found that alkaloidal extracts from the roots of *T. bakis* has antimalarial activity. Both *T. evansi* and *Plasmodium falciparum* are blood parasites. The toxicity of *T. bakis* revealed increase in the concentration of GOT, GTP and alkaline phosphatase. The increase in urea and creatinine was not significant. Histopathological changes showed necrotic foci in the liver. This result disagreed with the result obtained by Diallo *et al.* (1999) who found that the aqueous extract of *T. bakis* increased the biliary flow. It can be conducted that the effect of *T. bakis* depends on the type of the extract.

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