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Trypanocidal Effect of *Cannabis sativa* on Experimental Camel Trypansomosis

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ABSTRACT

Trypanosoma evansi causes camel Trypanosomosis. *Cannabis sativa* was evaluated *in vivo* against *trypanosoma evansi* experimentally infected in rats. Six groups of 6 rats each aged 6-8 weeks were used. Aqueous and methanolic extracts of the whole plant were administered orally at dose rates of 125 and 250 mg kg^{-1} BW for 10 consecutive days. The results were compared to the standard drug Trypacide (quinapyramine) . The parasitaemia in each rat was followed for 60 days. Both aqueous and methanolic extracts cleared the parasite on the second day of treatment for the dose 125 and 250 mg kg^{-1} BW. Relapse occurred at day 48 of treatment for both doses of methanolic extract. The pasitaemia appeared after 18 days of treatment with 125 mg kg^{-1} BW water extract. Trypacide, the standard drug curred the parasite for 10 days only were relapse occurred at day 11. The objective of this study is to evaluate trypanocidal activity of certain plant extracts compared to standard drugs.

Key words: *Cannabis sativa*, *Trypanosoma evansi*, trypacide

INTRODUCTION

Trypanosomosis caused by Trypanosoma spps are closely related to forms of diseases of man and animals (Soulsby, 1982). Camel Trypanosomosis is caused by *Trypanosoma evansi* and the disease is referred to as surra (FAO, 1988). Surra is of great economic importance in Africa, where thousands of animals die each year (Stephen, 1986). The disease is transmitted by the blood biting fly Tabanids. It causes emaciation, intermittent fever, weakness, anaemia and enlargement of lumph nodes, Saror (1980). Control of camel trypanosomosis is based mainly on treatment by trypanocidal drugs. The extensive use of these drugs resulted in the appearance of drug-resistant trypanosomes (El-Rayah *et al*., 1999). The situation was made worth by the slow development of new tryapanocidal drugs. This why an ethnobotanical approach collaboration with traditional healers remides may prove to be a rich source of drug discovery (Farnsworth *et al*., 1985). Herbal medicine is a common practice all over the world. Sudan is rich with plants used as herbal treatment. Elhardallou (2011) studied the cytotoxicity and biological activity of many Sudanese medicinal plants. In this study *Cannabis sativa* is selected upon its use in many countries for the treatment of, constipation, gout, malaria and absent-madness (Marijuana, 1975). *Cannabis* was used in the twentieth century B.C. in Egypt to treat sore eyes. In India, prior to tenth century B.C. bhanga was used as an anesthetic and anti phlegmatic (Sachindra and Pradhan, 1977). The plant

was used as an anesthetic in surgery in ancient China. *Cannabis* was also widely used in Indian medicine, in both the Hindu and Moslem systems of drugs.

MATERIALS AND METHODS

Rats: White albino rats were used in the present study. The rats were obtained from the central veterinary research laboratory, Soba. They were housed in laboratory cages, fed with pellets and were watered *ad libitum*.

The parasite was isolated from naturally infected camel at Alshowak, Algadarif estate. Infected blood was inoculated into a mouse for propagation. The blood of the infected mouse was cryopreserved in liquid nitrogen. The rapid matching wet-examination technique described by Herbert and Lumsden (1976) was used by examining a drop of mouse blood under the 40x magnification of a microscopic and counting the number of Trypanosoma in each field and matched with log figure obtained from the reference table. *Trypanosomes* were injected I/P at dose of 5×10^5.

The plant material:

***Cannabis sativa*:** *Cannabis sativa* is a member of the family *Cannabinaceae*. *Cannabis sativa* preparation is known by various names worldwide. It is called Marijuana in America. Bhang, Takrori in Tunisia, Habak in Turkey, Hashish in Middle East, (Sarpong, 1971). In Sudan, the most famous names of *Cannabis* preparations are bango and hashish. The name bango in Sudan may be derived from the Indian name bhang. *Cannabis sativa* is a mono specific plant, a shrub-type of plant with a strong fragrance and grows in different areas in the world. The length of the plant ranges between 30 cm to 6 m. Its leaves are week and toothed and cluster in a shape of a fan.

Preparation of the plant extract: The whole plant of *Cannabis sativa* were obtained from Niala, South Darfur, Sudan, cleaned and dried. The oil was extracted as follows:

- The powder of *Cannabis sativa* whole plant obtained was successively extracted with methanol for 4 h, using soxhelt apparatus. The extract was occasionally shaken during the first 4 h and was then filtrated. The filtrate was evaporated under vacuum and the residue is brownish in color. It is kept as stock for use. The aqueous extract was extracted by dissolving in distilled water and then put in water bath for half an h. The extract will be kept overnight and then filtrate and kept as stock

Standard drug: Trypacide or quinapyramine was manufactured by Rhone-Merieux, France, is used at dose rate of 3.0-5.0 mg kg^{-1} BW subcutaneously.

Experimental design: Groups of 6 rats each were used; they aged 4-6 weeks, weighted 125-150 g and divided as follows:

- **Group 1:** Infected untreated control
- **Group 2:** Infected ad treated with 10 mg kg^{-1} of trypacide S C^{-1}
- **Group 3:** Infected and treated with 125 mg kg^{-1} BW of plant methanolic extract
- **Group 4:** Infected and treated with 250 mg kg^{-1} of plant methanolic extract
- **Group 5:** Infected and treated with 125 mg kg^{-1} BW of plant aqueous extract+10 mg kg^{-1} BW trypacide

- **Group 6:** Infected and treated with 250 mg kg^{-1} of plant methanolic extract+10 mg kg^{-1} BW trypacide

The plant extract was given orally using nasogastric tube for 10 consecutive days.

RESULTS AND DISCUSSION

Trypacide was used as a standard drug in this experiment at a dose rate of 10 mg kg^{-1} BW. It was found that drug cured the parasite at the third day of treatment but relapse occurred after ten days of treatment. With the plant, it was clear that there was an immediate cure as from the second day of treatment when the methanolic extract was given at both doses. All the rats either given 125 or 250 mg kg^{-1} BW became aparasitaemic till day 48 when the parasite appeared with clearance percentage 100%. There was death in the group that given 125 mg kg^{-1} BW together with the standard drug and the percentage rate was found to be 90%. There was death associated with the untreated group with percentage rate 50% (Table 1).

Figure 1 presented comparison between the activity of the metabolic extract of *Cannabis sativa* and the standard drug trypacide and Fig. 2 presented comparison between the activity of the aqueous extract of *Cannabis sativa* and the standard drug trypacide. The best result was obtained with methanolic extract.

Table 1: Antitrypanosomal activity of *Cannabis sativa* extracts compared to trypacide

Group No.	Treatment	Dose used	Initial trypanosomes clearance	Relapse	Percentage of death
1	Infected untreated control		None		50% between day 40-45
2	Treated with trypacide	10 mg kg^{-1} BW	Day 4	Day 11	20% between 30-45 day
3	Treated with (M) extract	125 mg kg^{-1}	Day 2	Day 48	none
4	Treated with (M) extract	250 mg kg^{-1}	Day 2	Day 48	none
5	Treated with (A) extract+trypacide	125 mg kg^{-1}+10 mg	-		50% On Day 2
6	Treated with (A) extract+trypacide	250 mg kg^{-1}	Day 8	Day 18	90%

Each group was composed of 6 rats each. The parasite was given at a dose rate of 5×105. (M) represents methanolic extract and (A) represents aqueous extract

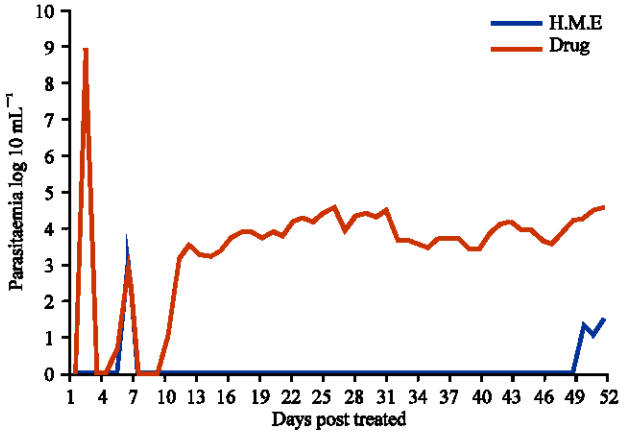

Fig. 1: Comparison between the activity of the methanolic extract of *Cannabis sativa* and the standard drug trypacide

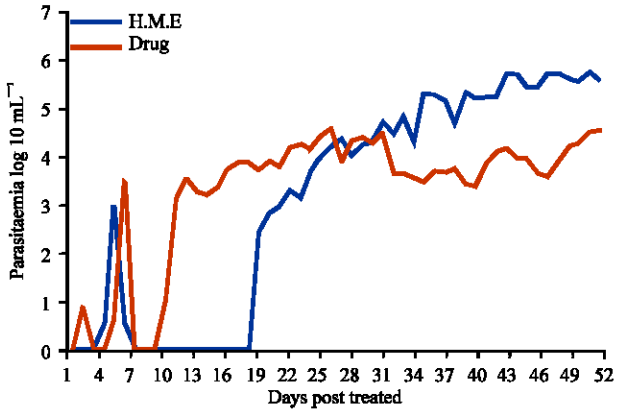

Fig. 2: Comparison between the activity of the aqueous extract of *Cannabis sativa* and the standard drug trypacide

The search for an active trypanocidal drug from a plant origin is a concern of many researchers. The study gave an indication of the activity of *Cannabis sativa* used in this study. No relapse occurred with both doses of the methanolic extract which indicates that the plant has a trypanocidal activity with slight toxicity associated with aqueous extract given together with the standard drug. The result obtained was agreed to that of result agreed with that of Nok *et al.* (1994) who studied the effect of *Cannabis sativa*, They found that the aqueous extract of the seeds of *C. sativa* when administered by injection at a dose of 50 mg kg^{-1} BW/day for five consecutive days, cured rats infected experimentally with *T. brucei*. The high level of activity displayed by the methanolic extract, indicates that the methanol might be capable to extract the biological active principle (s) responsible for the trypanocidal effect of the plants used.

Identifying bioactive compounds and establishing their health effects are active areas of scientific enquiry (Kris-Etherton *et al.*, 2004). Further study of the isolation and characterization of the plant should be applied to know the active components.

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