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ANTI-TRYPANOSOMAL ACTIVITY OF METHANOL EXTRACT OF *CHAMAECRISTA MIMOSOIDES* LEAF IN *TRYPANOSOMA BRUCEI* INFECTED MICE.

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ABSTRACT

Trypanosomiasis is a protozoan disease cause by a unicellular parasites and transmitted by the bite of tsetse fly. Pathogenic trypanosome infections of domestic animals in sub-Saharan Africa largely account for the low livestock productivity in the continent thus making it an important priority for the agricultural sector and biomedical and public agencies. Methanolic extract of leaves of Chaemacrista mimosoides was investigated for anti-trypanosomal activity, using mice infected with Trypanosoma brucei brucei. Five groups comprising of five mice per group were used in the study. Four groups were infected with T. brucei brucei using 0.5ml of donor blood corresponding to 2 parasites per microscope field. Group 1 was not infected and was used to monitor the cause of any disease, group 2 was infected but not treated and served as the negative control, group3 was infected and treated with 0.2ml of Berenil and served as the positive control while groups 4 and 5 were treated with 300 and 400mg/kg body weight of plant extract respectively. There was a significant dose dependent reduction ($p<0.05$) in the parasite count with the mean values of 4 and 3.6 parasite per microscope field observed in the dose of 300 and400mg/kg body weight of extract respectively compared with the positive control value of 8.75. The result of the study showed that the methanolic leaf extract of *chaemacrista mimisoides* possess trypanocidal properties and could serve as a source of new trypanocidal agent from medicinal plants.

KEYWORDS: Trypanosomiasis, Chaemacrista mimosoides, *Trypanosoma brucei brucei*, mice, Methanol leaf extract.

INTRODUCTION

Trypanosomiasis is a disease that results in annual losses in agricultural production of up to 4.5 billion U.S. Dollars in Africa alone (Adamu et al., 2009). The protozoan parasites that cause trypanosomiasis are *Trypanosoma brucei brucei*, *T. congolense*, *T. brucei gambiense* and *T. brucei rhodesiense.* The parasite is unicellular and transmitted by the bite of tsetse fly (Warren, 1988). Pathogenic trypanosome infections of domestic animals in sub-Saharan Africa largely account for the low livestock productivity in the continent thus making it an important priority for the agricultural sector and biomedical and public agencies (Aliyu et al., 2010). *Trypanosoma brucei brucei* is the most widely distributed of the pathogenic animal trypanosomes and is the most important single cause of economic losses in camel rearing causing morbidity of up to 30% camel rearing areas (Ngassoum et al., 2003). Studies have shown that the parasite can infect all species of domestic livestock. Five drugs (suramin, melasoprol, eflornithine,pentamidine and Berenil) are currently available for the treatment of trypanosomiasis (Adeiza et al., 2010) but these drugs are expensive, limited in availability and poorly distributed in rural areas and have also been shown to be toxic (Asuzu & Chineme, 1990). The need for the development of cheap, safe, effective and easy-to-administer drugs for the treatment of African trypanosomiasis have led to the study of plants that are traditionally said to have anti-trypanosomal effects and several medicinal plants have been reported as potent trypanocides (Atawodi et al., 2003; Mbaya et al., 2011).

The use of herbal remedies in the treatment of trypanosomiasis is potentially promising with some ethno medicinal plants used against the disease Have been demonstrated to show potent trypanocides. Also the exploitation of certain herbs and other plant materials said to be traditionally useful in the control of trypanosomiasis have also provided better and cheaper alternatives (Nok et al., 2005). *Chamaecrista mimosoides*formerly known as C*assia mimosoides*is an erect non branched, smooth, non woody herb or shrubby plant, 0.8-1.5 metres high. Leaves are pinnate and about 20cm long. Their flowers are yellow, 2cm long and borne on auxiliary and terminal racemes. Fruits are in pods, about 10cm long, 9cm wide, thickened and contains about 30 seeds. The leaf extract of *Chamaecrista mimosoides* have been found to contain important phytochemicals such as anthraquinones, carbohydrates,

glycosides, cardiac glycosides, steroids flavanoids, saponins, phytosterols, gum and mucilage (Colle et al., 2003) (Cordell, etal, 1991).

However the plant is yet to be significantly evaluated for efficacy against trypanosomal infections *in vivo*. This study reports the therapeutic activity of the methanolic leaf extract of *Chamaecrista mimosoides* in experimental *Trypanosomal brucei* infected mice.

MATERIALS AND METHODS

STUDY AREA

The experiments were conducted in the department of Biochemistry, Federal University of Technology Minna.

PLANT MATERIAL

Chaemacrista mimisoides leaves were obtained from Bida Niger state and identified by a plant taxonomist Mallam Muhammad of the Department of Biological sciences FUT Minna. The leaves were properly rinsed with distilled water to remove dirt and air dried for two weeks. The dried leaves were then milled using the grinding machine.

EXPERIMENTAL ANIMALS

Healthy white mice (*Musmusculus*) of both sexes of about 20-35g were obtained from the animal rearing unit of STEP-B FUT Minna. The animals were kept in well ventilated laboratory cages with 12 hours daylight and 12 hours darkness cycle. The mice were maintained on a commercial poultry feed (vital feeds, Kaduna Nigeria) and drinking water.

TEST ORGANISMS

Trypanosoma brucei brucei (Federa strain) was obtained from infected rats from the Nigerian Institute of Trypanosomiasis Research (NITR) Kaduna, Nigeria. Parasites harvested from the infected rats were used for the *in vivo* test and infection of the experimental animals.

EXTRACT PREPARATION AND PHYTOCHEMICAL SCREENING

The methanolic leaf extract of *Chaemacrista mimosoides* was prepared by soaking 100g of the powder in 600 ml of absolute ethanol and extracted using the hot reflux method. The preparation was then filtered using a whatmann filter paper**.** The filtrate was concentrated to dryness and stored at 4 degree Celsius until required.

Analysis of the major phytochemical constituents were carried out qualitatively according to the procedures of Odebiyi and Soforawa (1978).

IN-VIVO ANTI TRYPANOSOMAL ACTIVITY

Twenty five mice were used in the study. The mice were randomly grouped into five groups (1, 2, 3, 4 and 5) with each group containing five mice. Mice in groups 2-5 were inoculated intraperitoneally with 0.5ml of rat blood containing 2 parasites per field. The number of parasite were determined using the method described by Herbert and Lumsden (1976). After three days post infection, smear was taken when parasitaemia was established. The mice in groups 4 and 5 were treated with 300mg/kgbw and 400mg/kgbw of the leaf extract respectively while those in group 3 were treated with 0.2ml of Berenil (Diminazene aceturate). The mice in group 1 were left uninfected so as to monitor the cause of the disease and any other infection. The extract and drug were administered orally once daily for fourteen days and all treatment were terminated after that period. The animals were examined periodically for the presence of parasite.

POST PARASITE WEIGHT CHECK

The weight of the mice were measured before infection and after treatments.

PARASITAEMIA

On the third day post infection and after fourteen days of extract and drug administrations the parasitemia level of mice in the control and extract groups were checked. The tail of each mouse was pre-sterilized with a methylated spirit. The tail tip was cut to extrude blood and 2 drops of blood was dropped on a grease free microscope slide and viewed under the light microscope at ×40 magnification. The numbers of swarming parasite per microscope field was counted. For each parasitemia determination, five fields were examined by counting the numbers of parasite and the means were recorded.

HEMATOLOGICAL TESTS

The mice were slaughtered after conclusion of treatment and exactly 3ml of blood sample were collected from each animal. Hematological test was conducted.

STATISTICAL ANALYSIS

The data obtained were analyzed using the one way ANOVA and expressed as a mean ± standard error of mean. The difference between the mean were considered significant at $p<0.05$.

RESULT

The qualitative phytochemical analysis conducted indicates the presence of alkaloids, saponins, anthraquinones, phenols, flavonoids, tannins, steroids and glycosides. While phytochemicals like cardiac glycosides and terpenes were absent.

Table 1 shows the mean weight of the animals before infection and treatment and their weight post treatment. Group 1(uninfected) shows an increase weight during the time this research was conducted while group 2 to group 5 shows a decrease in weight. The most pronounced weight loss was observed in group 2 (infected but untreated). The infected mice also showed signs of weakness.

Table 2 shows the parasite count pre-treatment and post-treatment. There is an increase in the parasite level of mice in group 2, recording about 23.56 parasites per field. Animals treated with the standard drug had the lowest parasitaemia post infection, 2.50. There was a decrease in the parasite level of mice treated with the plant extracts. The mice treated with 400mg/kgbw of the plant extract had lower parasite count than those treated with 300mg/kgbw. This indicates that the methanolic leaf extract of *Chaemacrista mimisoides* is effective against *Trypanosome brucei brucei* at high dose.

Table 1: pre-treatment and post-treatment weight

Group	Pre-parasite weight (kg)	Post parasite & treatment weight (kg)
Uninfected	26.93 ± 0.67^{a}	30.59 ± 0.63^{c}
Infected &untreated	27.28 ± 0.50^{a}	20.08 ± 0.86^{ab}
Standard(Berenil)	28.39 ± 0.95^{a}	22.32 ± 0.97^{b}
300mg/kgbw extract	28.00 ± 1.40^{a}	21.37 ± 0.56^{ab}
400mg/kgbw extract	26.49 ± 0.76^{a}	19.72 ± 0.69^{a}

Table 2: pre-treatment parasitaemia and post-treatment parasitaemia

Groups	Pre-treatment parasitaemia	Post-treatment parasitaemia
Uninfected	nil	.nil
Infected & untreated	8.52±0.30	23.56±3.34
Standard(Berenil)	9.16±0.26	2.50±0.39

300mg/kgbw extract	8.68±0.14	3.87±0.40
400mg/kgbw extract	9.24±0.33	3.60±0.67

The hematological tests conducted is shown in table 3. The animals in the uninfected group has a higher packed cell volume than the other infected groups. The PCV, RBC and Hb of the infected animals is much lower than that of the uninfected animals while the WBC is higher in the infected mice. This indicates that the parasite *Trypanosome brucei* causes anaemia and affects the erythroblast. The presence of a large number of white blood cells shows that the animal's immune system has responded to the parasite by releasing the white blood cells to fight against the infection.

Table 3: Effect of methanol leaf extract of *Chaemacrista mimosoides* on hematological parameters of *T.brucei* infected Mice

Groups	PCV (%)	RBC($X10^{12}$/L)	WBC($X10^{9}$/L)	Hb (g/dL)
Uninfected	41.40±0.40	4.60 ± 0.04c	3.54 ± 0.04	13.20±0.20
Infected & untreated	28.50±0.50	2.56±0.19	4.70±0.50	9.30±2.02
Standard (Berenil)	37.00±0.40	2.45 ± 0.48	4.50±0.20	11.50±0.61
300mg/kgbw extract	37.66±0.66	2.62±0.60	4.26±±0.19	12.49±0.95
400mg/kgbw extract	38.33±0.33	3.10±0.57	4.70±0.20	12.41±0.58

DISCUSSION

The loss of weight observed in the infected groups is in accordance with the report of Mann et al (2008) in which infection with *T. brucei* was associated with weight loss in mice and rats. Infection with *T. brucei* also resulted into other ailments in various tissues and organs, anaemia, emaciation and eventually death. The reduction in RBC, PCV and Hb of infected animals shows that the plant extract couldn't stop the anemic action of the parasite. However, as the plant extract was administered, a reduction in WBC showed that the leaf extract was able to combat the parasite and the mice immune system had little need for its white blood cells. The result obtained from this investigation showed a dose dependent reduction in the parasite level of mice with the parasitaemia of mice treated with 300mg/kgbw of leaf extract of *Chaemacrista mimisoides* being 3.86 parasites per field while those treated with 400mg/kgbw had 3.60 parasites per field. The results indicates that the methanolic leaf extracts of *Chaemacrista mimisoides* contains phytochemicals which are active anti-trypanosomal agents against *T.brucei brucei*. A 50% reduction in parasitaemia is an indication of significant trypanocidal activity (carver, 1973). The mechanism by which these extracts exhibit trypanocidal action was not determined. However, Sepuveda and Cassels (1996) suggested that many natural products exhibit their trypanocidal activity through

interference with the redox balance of the cellular defence against oxidative stress. This is because natural products possess structures capable of generating radicals that may cause peroxidative damage to alterations in redox balance. It is also known that some agents act by binding with the kinetoblast DNA of the entire parasite.

CONCLUSION

The results obtained from this research indicates that the methanolic leaf extract of *Chaemacrista mimisoides* possesses anti-trypanosomal activity.

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