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Assessment of Effects of Some selected Herbs and combinations on *Trypanosoma brucei* Infected Albino Rats

Olatunde[1]*, O.A., Jegede[2], H.O., Ameen[3], S.A. and Belewu[4], M.A.
[1]Department of Theriogenology and Production, Faculty of Veterinary Medicine, University of Ilorin, Nigeria
[2]Veterinary Teaching Hospital, University of Ilorin, Ilorin, Nigeria.
[3]Department of Veterinary Medicine, Faculty of Veterinary Medicine, University of Ilorin, Nigeria.
[4]Department of Animal Production, University of Ilorin, Ilorin, Nigeria.

***Corresponding author:** tundeola@unilorin.edu.ng, +2348033953880

Abstract

Trypanosomiasis is one of the major constraints to the livestock industry and food security in Sub-Saharan Africa with an estimated direct loss between $600 million and $1.2 billion annually on a global scale. Therefore, this study aimed at evaluating the trypanotolerant effects of selected herbs and combinations on Trypanosoma brucei infected albino rats. Rats infected with Trypanosoma brucei were subjected to 5 herbal diet treatments and data collected on temperature, pulse rate, feed intake, and weight gain. Results obtained showed no significant difference in the rectal temperature across all treatments (p>0.05). However, results on pulse rate showed that rats placed on Diets E, F, and Control Diet A had significantly higher pulse rate ($p<0.05$) compared to other diets while rats fed Diet C presented significantly lower pulse rate ($p<0.05$). Rats placed on Diet C showed higher ($p<0.05$) average feed intake and overall acceptance while rats fed Diet D showed by far better weight gain. The rats preferred single bitter plant spices in the dieter than mixture combinations, where the former had better acceptability and performance during the study. Rats fed with Vernonia amygdalina had better feed intake probably due to palatability. Also, rats fed Occimum gratissimum alone in their diet had a better feed conversion ratio with moderate physiologic response to the disease suggesting that the plant has trypanotolerant properties.

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Keywords: Herbal diets, albino rats, *Trypanosoma brucei*, weight gain and feed conversion

Introduction

The fourth tropical disease that must be treated, controlled, and eradicated for effective performance and sustenance of animal production is Trypanosomiasis (WHO, 2005). The search for drugs and formulations that are safe, affordable, and effective against both the early and late stages of the diseases is very essential (Biobaku *et al*., 2008). Chemotherapy of Sleeping sickness is unsatisfactory because only four drugs of which three were developed over fifty years ago are currently approved for the treatment of the disease (Biobaku *et al*., 2009). In addition, these drugs display undesirable toxicity and the emergence of drug-resistant trypanosomal strains (Matovu *et al*., 2001). African Trypanosomiasis or sleeping sickness is a great scourge to man and domestic animals in Africa (Cox, 2004), and the use of rats to study the rate of infection and their response to herbal-based diets will be a guide to the use of herbs in the prevention and treatments of the disease. There is relatively no information on the effect of herbal diet on the performance of trypanosome infected animals like rats; therefore, the aim of this work was to evaluate the effect of herbal diet on the performance of trypanosome infected rats.

Materials and Methods

Experimental Animals

Twenty-one (21) albino rats were used to study the response of the animals to an herbal-based diet. The albino rats were obtained from the Department of Biochemistry, Faculty of Science, University of Ilorin, and Faculty of Veterinary Medicine, University of Ibadan, Ibadan. The rats were kept in a wooden cage with three (3) rats per cage; feed and water were given ad libitum for a period of two (2) weeks after which eighteen rats were infected with the T. brucei parasite

Preparation of the Inoculum

A monomorphic strain of clone number FE/p8/no. l T. brucei was used in the experiment. The strain was isolated at Nigerian Institute for Trypanosomiasis Research (N.I.T.R) Vom, Jos. The blood samples from the tail of infected mice were collected by the use of a 2ml syringe. Animals used in this experiment were infected by syringe passages intraperitoneally.

Inoculation of Rats with Trypanosome

The rats were divided into seven groups (A- G) with each group containing 3 rats and group A served as a control. Blood obtained from the tail vein of the infected rat was diluted with 0.855ml of physiological saline solution. This was drawn into a syringe and 18 rats were injected. Each receiving, 0.2ml of the diluted blood intraperitoneally which was equivalent to 3x 103 of T. brucei .

Preparation of plant spices

The plant samples (leaves from *Citrus aurantifolia,Vernonia amygdalina, Occimum gratissimum, Citrus aurantifolia + Vernonia amygdalina,Citrus aurantifolia + Occimum gratissimum, Occimum gratissimum + Vernonia amygdalina)* were collected within the University of Ilorin main campus while others were bought from the local market in Ilorin metropolis, Nigeria. The samples were identified at the Department of Plant Science, University of Ilorin, Nigeria. The samples were washed in a running tap to remove soil and dust particles and later air-dried on the laboratory bench for five days. The dried samples were milled with pestle and mortar into a powdery form. The powdered samples were stored in dry, clean containers with lids. These were mixed with prepared diets at a 5% inclusion rate as shown in Table 1. The diets were fed to the experimental rats for a period of 30 days.

Table 1: Composition of the Experimental Diet on as fed basis (%)

Ingredients %	A	B	C	D	E	F	G
Maize	55.00	50.00	50.00	50.00	50.00	50.00	50.00
Plant Extract	-	5.00[b]	5.00[c]	5.00[d]	5.00[e]	5.00[f]	5.00[g]
Soya bean meal	25.00	25.00	25.00	25.00	25.00	25.00	25.00
Brewer Dried Grain(BDG)	19.00	19.00	19.00	19.00	19.00	19.00	19.00
Vitamin-Mineral Premix	0.50	0.50	0.50	0.50	0.50	0.50	0.50
Salt	0.50	0.50	0.50	0.50	0.50	0.50	0.50
Total	100.00	100.00	100.00	100.00	100.00	100.00	100.00

Note: b-g; Plant extracts: Vit/ Min Premix is Bio-organic Inc source.

b- *Citrus aurantifolia,* c- *Vernonia amygdalina* , d- *Occimum gratissimum*

e- *Citrus aurantifolia* + *Vernonia amygdalina*, f- *Citrus aurantifolia* + *Occimum* *gratissimum*, g- *Occimum gratissimum* + *Vernonia amygdalina*

Parameters evaluated

In the course of the experiment, data were collected on the parameters: Feed intake, pulse rate (beat/min), rectal temperature (OC), weight gain (BWG) and feed conversion ratio (F/G).

Statistical analysis

Data collected were subjected to analysis of variance (Steel and Torrie, 1980) with treatment means separated (Duncan, 1955).

Results

The results obtained (Table 2) showed no significant difference in the rectal temperature across all treatments (p>0.05). However, results obtained on pulse rate showed that rats placed on Diet E (*Vernonia amygdalina* + *Citrus aurantifolia);* Diet F (*Occimum gratissimum* + *Citrus aurantifolia*) and Control Diet A had significantly higher pulse rate ($p<0.05$) (387.2, 385.3, 388.5 respectively) compared to other diets. This was followed by rats placed on Diet G (*Occimum gratissimum* + *Vernonia amygdalina)* (375.1) and sole *Citrus aurantifolia* Diet B (377.50) though numerically (p>0.05). Rats placed on Diet with *Vernonia amygdalina* treatment had significantly lowered pulse rate ($p<0.05$).

The Highest weight gain was observed in the rats placed on Diet D *(Occimum gratissimum)* ($p<0.05$), while those placed on Diet E *(Vernonia amygdalina* + *Citrus aurantifolia)* and F (*Occimum gratissimum* + *Citrus aurantifolia*) herbal mixtures showed significantly lower weight gain compared to other diets ($p<0.05$). Rats placed on Diet C (Vernonia amygdalina) showed higher average feed intake ($p<0.05$) compared to both Diet B (*Citrus aurantifolia*) and D (*Occimum gratissimum).* Average daily weight gain was the lowest on the Control A ($p<0.05$). There was however no significant difference in weight gain for rats fed Diet B, E, F and G (p>0.05). Feed conversion rate was best ($p<0.05$) for diet D (1.06) while worst feed conversion ratio was measured for Diet E (1.54). Trypanocidal activity of all herbs was not assessed.

Discussion

Result of the study showed that rats infected with Trypanosome did not display any change in body temperature (pyrexia) characteristic of trypanosomal infection as reported by Zwart *et al.* (1991). The lack of pyrexia could be as a result of the innate immune system of the rats to regulate the production of pro-inflammatory cytokines and by the production of type II anti-inflammatory cytokines IL-4, IL-10, IL-13 (Namangala *et al.*, 2009). Host cells initiate acute inflammatory response which is beneficial at first (Janeway and Medzhitor, 2002; Takeda *et al.*, 2003) but when sustained can cause pathology (cytokine storm). Hence, it is suggestive that such a stabilizing and pathogen-arrestive mechanism is taken up by the rat's immune system in order to suppress the trypanosome invasion.

Table 2: Effects of Selected Herbs on Performance Indices of Albino rats.

Treatment	Rectal Temp. (^{0}C)	Pulse rate Cycle/minute	Feed intake (g/day)	Weight gain (g/day)	Feed conversion ratio F/G
A	37.55	388.50^a	17.12^c	14.91^e	1.15^b
B	37.65	377.50^b	24.77^b	17.22^c	1.44^c
C	37.65	328.50^d	26.19^a	17.16^b	1.53^d
D	37.65	357.50^c	18.64^c	17.54^a	1.06^a
E	37.55	387.20^a	25.55^{ab}	16.55^c	1.54^d
F	37.60	385.30^a	21.68^{bc}	16.49^c	1.31^{bc}
G	37.65	375.10^b	25.52^b	16.67^d	1.53^d
SEM (±)	0.57	3.51	0.18	0.31	0.04

Means in columns with different superscripts are significant ($p<0.05$)

A: Control, B: *Citrus aurantifolia*, C: *Vernonia amygdalina*, D: *Occimum gratissimum*, E: *Vernonia amygdalina* + *Citrus aurantifolia*, F: *Occimum gratissimum* + *Citrus aurantifolia*, G: *Occimum gratissimum* + *Vernonia amygdalina.*

The diminishing pulse rate may be caused by the phytochemicals present in the herbs that are known to interfere with body metabolic functions and electron transport chain (Sepulveda-Boza and Cassels, 1996). Moreover, the phytochemicals could act as immunostimulants that significantly delays parasitaemia and increases in survival time in mice (Morrison and Murray, 1979). This is especially true for herbs and natural products containing alkaloids, terpenes, polyphenols, and many more than they act as a potent growth inhibitor of trypanosomes (Wright and Philipson, 1990) and possess anti-trypanosomal activity (Hoet *et al.,* 2007). Some plant extracts have been reported to contain potent trypanocidal constituents (Atawodi, 2005: Biobaku *et al.*, 2008). Aqueous and methanol extracts of stem bark of *Khaya senegalensis* have been shown to possess in vitro activity against T. brucei (Wurochekke and Nok, 2004; Atawodi, 2005).

The results reported on performance revealed that the leaves of the herbs also contain some water and ethanol-extractable phytochemicals that possess in vitro activities against T. brucei as observed in the average weight gain index. The observed in vivo anti–T. brucei activity of the stem bark ethanol extract of these plants supports earlier reports that some plant extracts possess in vivo activities against trypanosomes (Iroanya *et al.*, 2009; Biobaku., 2010). This could also provide the scientific basis for the traditional use of herbs such as K. senegalensis in the management of trypanosomiasis (Atawodi *et al.*, 2002). The exact mechanism of action for this extract is unknown since the active ingredient(s) were not isolated. However, previous reports attributed the trypanocidal activity of a number of tropical plants to the flavonoids (azanthraquinone), highly aromatic planar quaternary alkaloids, barbarine and harmaine (Hopp *et al.*, 1976). Furthermore, Sepulveda-Boza and Cassels (1996) suggested that many natural products exhibit their trypanocidal activity through interference with the redox balance of the parasites acting either on the respiratory chain or on the cellular defenses against oxidative stress. This is because natural products possess structures capable of generating radicals that may cause peroxidative damage to trypanothione reductase that is very sensitive to alterations in redox balance. It is also known that some agents act by binding with the kinetoplast DNA of the parasite (Atawodi *et al.*, 2003). The ethanol leaf extract of O. gratissimum was found effective in inhibiting/preventing disease conditions after infection through immunostimulatory mechanisms (Oladunmoye, 2006), this might be the reason the rats had an excellent feed conversion during the study despite being challenged with T. brucei.

In terms of feed intake, rats fed the herbal mixture diets tended to eat less compared to the control and sole-herb diets, especially Diet D (Occimum gratissimum treatment) which showed superior feed intake by rats. This is suggestive of the cross-interference of phytochemicals in the herbal mixtures which could hamper feed preference by rats and subsequently resulted in a fall in body weight, which was not the case for Diet D which showed an impressive feed conversion rate. Rats placed on Diet C (Vernonia amygdalina) showed a remarkable increase in weekly body weight gain, probably due to the better level of feed intake observed and the preference and palatability of the herb (Ogbadoyi *et al.*, 1999 and Tijani *et al.*, 2009).

It was therefore concluded that rats preferred the sole combination of bitter plant spices than mixed combinations for better acceptability and performance during the experimental period. Also, rats fed sole Occimum gratissimum diet had better average weight gain, better feed conversion with the moderate physiologic response to the disease suggesting that the plant has trypanotolerant properties.

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