DOSE RESPONSE AND AMELIORATIVE EFFECTS OF RESVERATROL ON TRYPANOSOMOSIS-INDUCED REPRODUCTIVE TOXICITY IN DIMINAZENE ACETURATE TREATED *TRYPANOSOMA BRUCEI* INFECTED MALE DOGS

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ABSTRACT
The study was done to assess the dose response and ameliorative effects of resveratrol on trypanosomosis induced testicular changes in diminazene aceturate (DA) treated *Trypanosoma brucei* infected male dogs. Thirty male dogs were used for the study and were assigned into six groups (n=5). Group A was infected with *T. brucei*, not treated with (DA) and not supplemented with resveratrol. Group B was presupplemented with resveratrol (50 mg/kg) for 7 days, Infected with *T. brucei* and treated with DA (7 mg/kg). Group C was presupplemented with resveratrol (100 mg/kg) for 7 days, infected with *T. brucei* and treated with DA (7 mg/kg). Group D was presupplemented with resveratrol (200 mg/kg) for 7 days, infected with *T. brucei* and treated with DA (7 mg/kg). Groups B, C and D were postsupplemented with 50, 100 and 200 mg/kg of resveratrol for 14 days respectively. Group E was infected with *T. brucei* and treated with DA (7 mg/kg). Group F was the control
There were significant decreases ($p<0.05$) in the parasitaemia level and rectal temperature of dogs in group C when compared to other supplemented groups. There were significant increases ($p<0.05$) testosterone level, testicular weight and epididymal sperm reserve of dogs in group C when compared to other supplemented groups, DA alone and the infected untreated group.
In conclusion, this study indicated that resveratrol at the dose of 100 mg/kg may constitute a promising compound for the amelioration of male reproductive toxicity in DA treated *Trypanosoma* infected male dogs.
Keywords: Trypanosomosis, reproduction, diminazene aceturate, resveratrol

INTRODUCTION
Trypanosomosis is a protozoan disease caused by pathogenic blood parasites known as trypanosomes of the genus *Trypanosoma*. It is transmitted by tsetse flies of the genus *Glossina*. The disease is unique to Africa, affecting both human (human African trypanosomosis or sleeping sickness) and animals (animal African trypanosomosis or Nagana) (Radostits *et al*., 2007). The main species of importance in livestock are *T.vivax, T. Congolense* and to a lesser extent *T. brucei* causing Animal African Trypanosomosis (AAT) (Steverding, 2008).

Its common clinical manifestations are weight loss, anaemia, anorexia, lethargy, weakness and dullness. It has been reported that trypanosomosis causes severe reproductive disorders characterized by decrease in testosterone level and testicular degeneration in males (Sekoni *et al*., 2004; Raheem *et al*., 2009). Oxidative stress induced by trypanosome is the major cause of testicular damage in trypanosomosis, due to the overproduction of reactive oxygen species (Adamu *et al*., 2006; Steverding, 2008).
Resveratrol is a natural polyphenol found in nuts, grapes and red wine (Siemann and Creasy, 1992). It is known for its anti-inflammatory, antioxidant, analgesic, cardioprotective, anti-aging and neuroprotective roles (Giovannimi *et al*., 2001; Sinha *et al*., 2002; Bradamante *et al*., 2004). Valenzano *et al*., 2006 reported that resveratrol is able to decrease germ cell apoptosis in mice and rats.

MATERIALS AND METHODS
Trans-resveratrol of analytical grade was obtained from Candlewood Stars Incorporated, Danbury, USA and carboxymethylcellulose was obtained from the Department of Veterinary Physiology and Pharmacology, Faculty of Veterinary Medicine, University of Nigeria, Nsukka. Trans-resveratrol, due to its low solubility in water, was suspended in 10 g/L carboxymethylcellulose.

Trypanosome brucei was isolated from a dog with natural infection presented to Veterinary Teaching Hospital, University of Nigeria, Nsukka and experimental animals were infected intraperitoneally at a dose of 1.5×10^6 trypanosomes per milliliter of saline diluted infected blood.

Experimental design

Thirty male dogs were used for the study and were assigned into six groups (n=5). Group A was infected with *T. brucei*, not treated with (DA) and not supplemented with resveratrol. Group B was presupplemented with resveratrol (50 mg/kg) for 7 days, Infected with *T. brucei* and treated with DA (7 mg/kg). Group C was presupplemented with resveratrol (100 mg/kg) for 7 days, infected with *T. brucei* and treated with DA (7 mg/kg). Group D was presupplemented with resveratrol (200 mg/kg) for 7 days, infected with *T. brucei* and treated with DA (7 mg/kg). Groups B, C and D were postsupplemented with 50, 100 and 200 mg/kg of resveratrol for 14 days respectively. Group E was infected with *T. brucei* and treated with DA (7 mg/kg). Group F was the control

Determination of parameters

Parasitaemia was monitored using the wet mount method daily after infection, daily after establishment, daily after treatment for clearance and weekly post clearance up to 10 weeks for relapse. The numbers of infective trypanosomes were determined using the rapid matching method of Herbert and Lumsden (1976). Rectal temperature was determined using a digital clinical thermometer. Enzyme Linked Immunosorbent Assay Test Kit (Monobind Inc., Lake Forest, CA 92630, USA) was used for the determination of serum testosterone levels according to the method of (Dorfman and Shipley, 1956). The dogs were orchiectomized and lignocaine was the local anaesthetic used (2 mg/kg). The testicles from each dog were carefully dissected out, trimmed free of extraneous tissues and weighed with a weighing balance. Epididymal sperm reserve was determined using the haemocytometer method described by Amann, 1986.

RESULTS AND DISCUSSION

The significant decrease ($p < 0.05$) in the parasitaemia level of dogs supplemented with 100 mg/kg of resveratrol when compared to those supplemented with 50 mg/kg, 200 mg/kg and the infected unsupplemented groups suggests that resveratrol at the dose of 100 mg/kg has optimum protective effect against early establishment of trypanosomosis

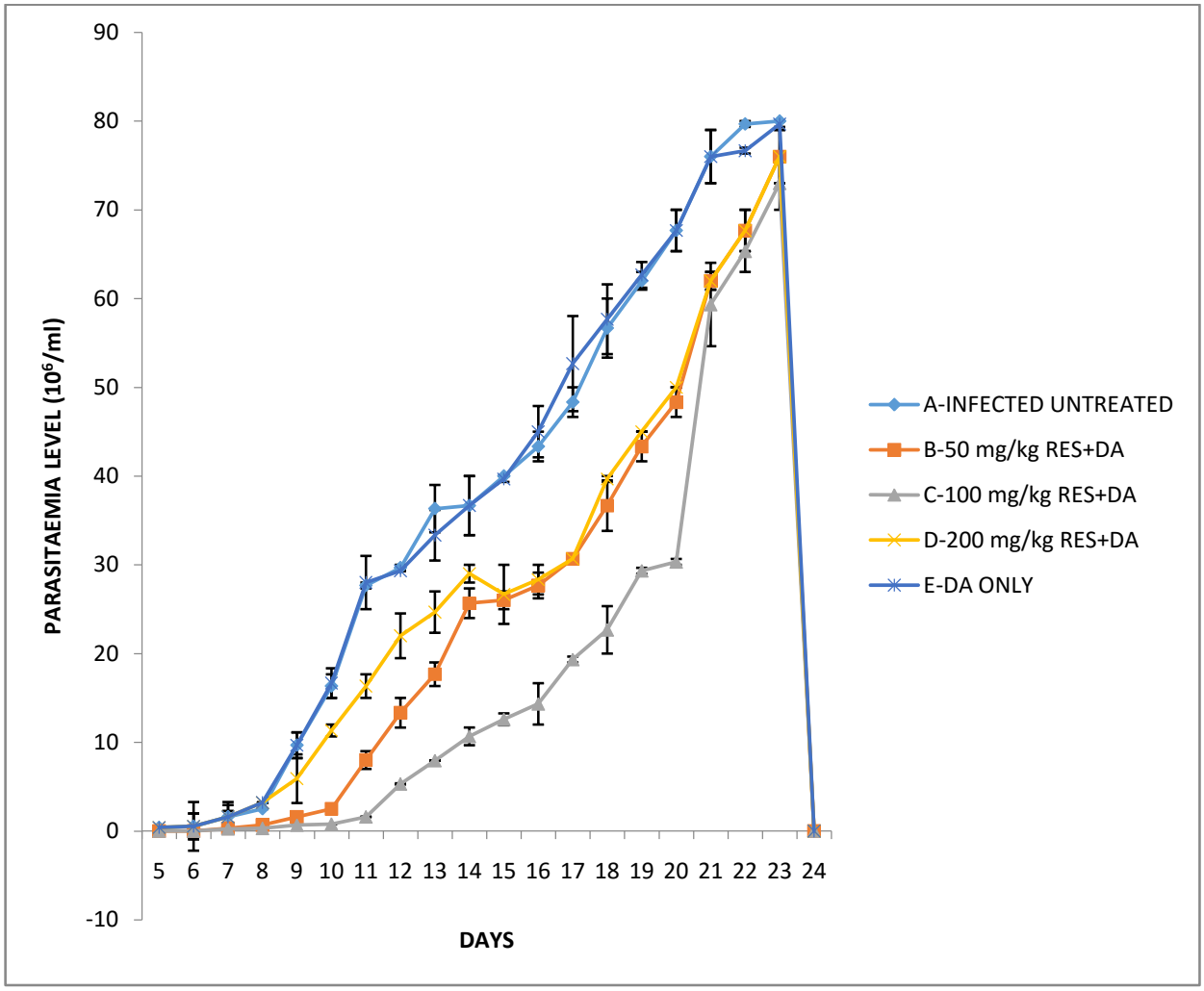


Figure 1: Effects of graded doses of resveratrol on parasitaemia levels of *T. brucei* infected male dogs
Key: RES – resveratrol, DA – diminazene aceturate

The significant increase ($p < 0.05$) in testosterone levels of dogs supplemented with 100 mg/kg of resveratrol when compared to those supplemented with 50 mg/kg, 200 mg/kg and the infected unsupplemented groups (Figure 2) may be that 100 mg/kg of resveratrol had a stimulatory effect on the secretion of gonadotrophins, the major endocrine regulators of spermatogenesis. The concentrations of FSH, which acts within the tubules to stimulate spermatogenesis, and LH, which signals the production of testosterone in Leydig cells have been reported to be enhanced by resveratrol (Juan *et al*., 2005)

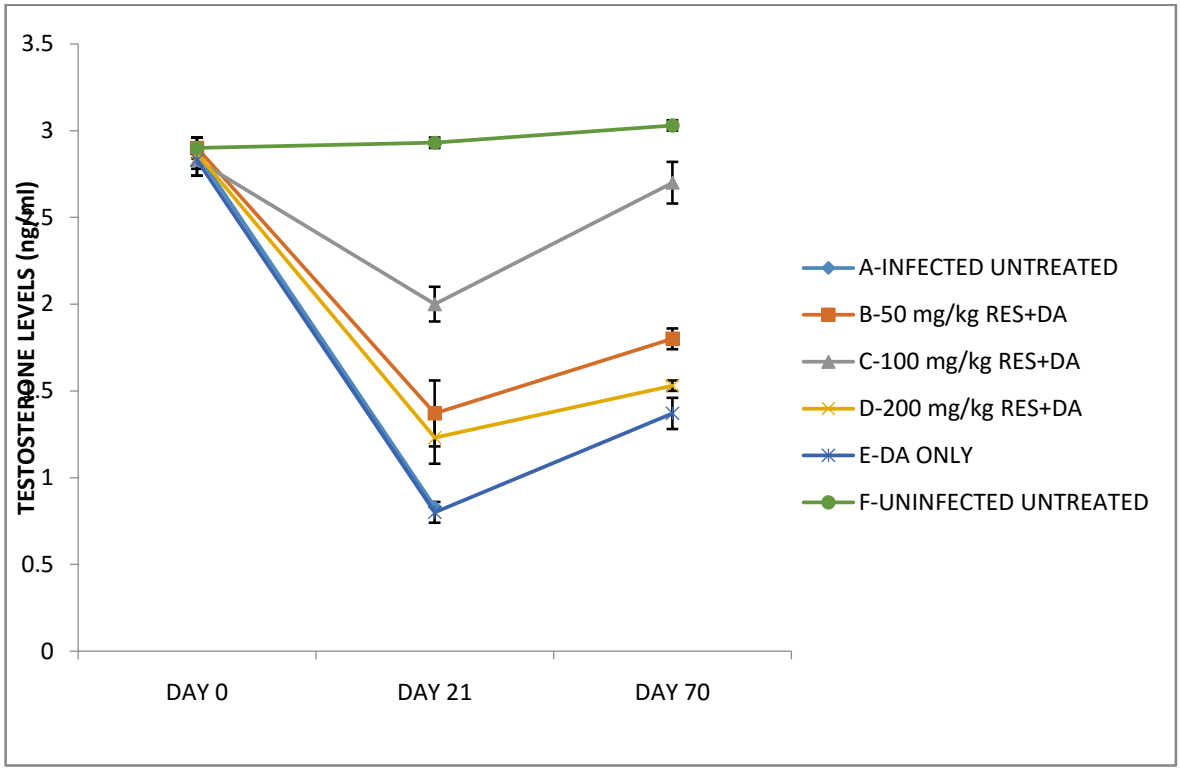


Figure 2: Effects of graded doses of resveratrol on testosterone levels of *T. brucei* infected male dogs
Key: RES – resveratrol, DA – diminazene aceturate

The significant increases ($p < 0.05$) in testicular weight and epididymal sperm reserve of dogs supplemented with 100 mg/kg of resveratrol and treated with DA when compared to those supplemented with 50 mg/kg and treated with DA, 200 mg/kg and treated with DA, treated with DA alone and the infected untreated (Table 1) may be due to significant increase in the testosterone level of 100 mg/kg supplemented group. It may also be due to antioxidant activity of resveratrol (Giovannimi *et al.*, 2001). Oxidative stress induced by trypanosome is the major cause of testicular damage in trypanosomosis, due to the overproduction of reactive oxygen species (Adamu *et al.*, 2006; Steverding, 2008; Losos and Ikede, 2012).

Resveratrol at the dose of 200 mg/kg produced negative effects in all the parameters determined which may be as a result of overdose. Resveratrol is an antioxidant and antioxidants at high concentrations have the opposite effect and act as pro-oxidants (Ramadori *et al.*, 2009; Minikawa *et al.*, 2011).
In conclusion, the findings from this study indicated that resveratrol, a phytochemical at the dose of 100 mg/kg may constitute a promising new compound for the amelioration of male reproductive toxicity in diminazene aceturate treated *Trypanosoma* infected dogs.

Table 1: Effects of resveratrol and DA on testicular weight and epididymal sperm reserve of *T.brucei* infected male dogs

Parameters	**Groups**					
	A-Infected untreated	B-50 mg/kg RESV+DA	C-100 mg/kg RESV+DA	D-200 mg/kg RESV+DA	E-DA only	F-Uninfected untreated
Testicular weight (g)	2.87 ± 0.06^{a}	3.26 ± 0.06^{b}	4.16 ± 0.13^{c}	3.06 ± 0.04^{b}	3.14 ± 0.12^{b}	3.34 ± 0.06^{c}
Epididymal sperm reserve (10^6/ml)	44.61 ± 3.17^{a}	122.59 ± 2.79^{b}	216.97 ± 8.91^{c}	100.61 ± 5.77^{d}	97.89 ± 4.05^{d}	221.93 ± 7.18^{c}

a, b, c, d - Different supercripts indicating difference between the mean ($p < 0.05$)

RECOMMENDATIONS

The findings from this study, encourages the use of moderate quantity of natural food stuff rich in resveratrol in preparing dog food.

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