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The beneficial effects of resveratrol supplementation on parasitemia, oxidative stress and serum biochemical parameters in *Trypanosoma brucei* infected dogs

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Abstract

This study investigated the effect of resveratrol supplementation on parasitemia, oxidative stress and serum biochemical changes in the *Trypanosoma brucei* infected dogs. The mean pre-patent period (MPP) of infection was 5.75±0.96 (5-7) days and 9.00 ± 0.82 (8-10) days between infected un-supplemented and supplemented respectively, which differed significantly ($p<0.05$). There was a significant increase ($P<0.05$) in the activities of malondialdehyde (MDA) post infection (pi) on the infected untreated group compared with the infected treated groups and the control. The mean serum alanine aminotransaminase (ALT) and aspartate aminotransferase (AST) were significantly ($P>0.05$) higher than the infected treated groups. The mean ALT of infected treated with both resveratrol and diminazene aceturate (DA) was significantly ($P<0.05$) lower than other infected treated groups. The mean creatinine and blood urea nitrogen (BUN) levels were significantly ($P<0.05$) higher in the infected untreated group. The creatinine level of the infected treated with both resveratrol and DA was significantly ($P<0.05$) lower than other infected treated groups, whereas the BUN did not differ significantly ($P>0.05$) within the infected treated groups when compared with the control. The mean albumin and total protein were significantly ($P<0.05$) higher in the infected untreated group, but the infected treated groups did not show any significant ($P>0.05$) difference. However, the total protein was significantly ($P<0.05$) higher in the infected treated with resveratrol than in other infected groups. It was concluded that pretreatment with resveratrol is beneficial in managing *T. brucei* infection, as it delays the onset of parasitemia and restores damages done to the liver and kidneys when combined with diminazene aceturate.

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Introduction

African animal trypanosomosis is a debilitating disease of humans and animals, often caused by *T. brucei*, and *T. congolense* in dogs (1,2). The disease is widespread and essential in Africa and sub-saharan African countries and affects a wide range of animals (3,4), leading to massive impact on economic losses, not only in dogs but in other animals as well, manifested as mortalities, reduced meat and milk yield in food animals and cost of treatment (5). Oxidative stress and inflammatory reactions have been suggested to play significant roles in the pathogenesis of trypanosomosis (6,7). Free radicals and many peroxides generated by trypanosomes and activated by mononuclear

phagocytes predispose cells to early aging, organ damage and death (8,9). Current trypanosomosis treatment and control strategies pose many challenges, because they depend on chemotherapy (9). The chemotherapy of trypanosomosis is characterized by drug toxicity, parasite resistance, and the parasite's ability to survive in cryptic areas poorly accessible to drugs, especially the brain (10,11). The absence of new curative drugs has further complicated the situation. The drugs have been combined with other therapeutic agents to ensure the optimal therapeutic performance of the available trypanocides and reduce toxicity (12-14). Several workers have reported using of antioxidants to enhance the efficacy of diminazene aceturate (15,16), though the result of antioxidants alone is gratifying, but not effective in eliminating the parasite (14). Resveratrol has been demonstrated as a potent and powerful antioxidant associated with antiaging, anticancer, anti-inflammatory effects and slows down a wide variety of illnesses (17,18). Recent studies have shown the quest, for searching a better way of managing trypanosomosis in animals and reduce the toxicity caused by the commonly used trypanocides.

Therefore, this work was designed to access the ameliorative effects of combination therapy of resveratrol supplementation and diminazene aceturate on oxidative stress and organ damage in *T. brucei* infected dogs.

Materials and methods

Experimental Animals

A total of twenty male dogs between the ages of 6 and 12 months were used for the study. The dogs were purchased from the Orba market in Enugu State, Nigeria. On arrival at the animal house, they were allowed to acclimatize before the commencement of the experiment. The blood and fecal samples were collected and examined for the presence of haemo and gastrointestinal-parasites. Animal studies complied with the ethical procedure of the Animal Use and Care Committee, College of Veterinary Medicine, Michael Okpara University of Agriculture, Umudike, which corresponds with NIH guidelines (19).

Experimental procedure

The dogs were acclimatized for two weeks before the commencement of the study, and the animals were grouped into five of four dogs each. Dogs in group I (Control) were uninfected and un-supplemented with resveratrol. Group II was infected, un-supplemented, and untreated. While groups III, IV and V were infected, un-supplemented with diminazene aceturate (DA) treated, infected with resveratrol-supplemented, and untreated with diminazene aceturate (DA), and infected with resveratrol-supplemented, and diminazene aceturate (DA) treated respectively.

Resveratrol and Diminazene aceturate administration

Ninety-nine percent pure resveratrol powder (Candlewood Star Incorporated Danbury, Connecticut, USA) was for the study. Due to its low solubility in water, trans-resveratrol was suspended in 10 g/L of carboxymethylcellulose (CMC) and administered orally at 100mg/kg body weight. Groups IV and V were pre-treated with resveratrol seven days before infection and fourteen days post-infection. Groups III and V were treated on day ten post infection (PI) (peak parasitemia) with diminazene aceturate at 7mg/kg body weight. The control group received ten g/L of (CMC) orally.

Trypanosome infection

T. brucei were obtained from the Department of Parasitology and Entomology, University of Nigeria, Nsukka. The trypanosome was first inoculated into a donor dog, before being used for infection of the experimental animals intraperitoneally (ip) at a dose of 1.5×10^6 trypanosomes per milliliter of saline diluted blood. The number of infective trypanosomes was determined using the rapid matching method of Herbert and Lumsden (20). Parasitemia was monitored in each infected dog daily from day two to patency.

Blood collection

About 5mls of blood was collected from each dog to monitor parasitemia, serum biochemical analysis and oxidative stress markers. The blood was put into a clean EDTA bottle, for parasitemia monitoring. The remaining 4mls of blood was put into a clean dry glass for serum biochemistry analysis and oxidative stress markers. These tubes were allowed to stay for some time to enhance serum yield and was later centrifuged at 2000rpm for 10 min to separate the sera from the cells.

Malondialdehyde (MDA) was determined according to Fauziah *et al.* (21). Catalase (CAT) activity was determined according to Iwase *et al.* (22). Reduced glutathione (GSH) was estimated as described by Hamid *et al.* (23). Serum superoxide dismutase (SOD) activity was measured according to Gaeta *et al.* (24). While, total serum protein, albumin, urea, creatinine, alanine aminotransaminase (ALT), aspartate aminotransferase (AST) and alkaline phosphatase (ALP) were determined using commercial kits (Randox) according to the manufacturer's instruction.

Statistical analysis

Data obtained from the study were analyzed using the one-way analysis of variance (ANOVA), and variant means separated by Duncan's multiple range tests, in SPSS version 20. A Significant difference was accepted at a probability level of $P \leq 0.05$.

Results

The mean pre-patent period (MPP) of infection was 5.75 ± 0.96 (5-7) days and 9.00 ± 0.82 (8-10) days between infected un-supplemented and supplemented respectively, which differed significantly (P<0.05). The level of parasitemia was significantly different (P<0.05) between supplemented and un-supplemented infected groups on days 9 and 10 (Table 1). There was a significant increase (P<0.05) in the activities of MDA pi on the infected untreated group compared with the infected treated groups and the control. The MDA of the resveratrol and DA treated group was significantly (P<0.05) lower than other infected treated groups when compared with the control (Figure 1). Then mean serum reduced glutathione (GSH), superoxide dismutase (SOD), and CAT activities were significantly (P<0.05) lower in the infected untreated group comparable with the control. In contrast, the activities were significantly (P<0.05) higher in the resveratrol and DA treated group than other infected treated groups (Figures 2-4). However, the mean GSH and SOD were significantly (P<0.05) higher in the DA treated group than the resveratrol treated group. The mean CAT activities did not differ significantly (P>0.05) between the DA and resveratrol treated groups. The mean ALT and AST were significantly (P>0.05) higher than the infected treated groups. The mean ALT infected treated with both resveratrol and DA was significantly (P<0.05) lower than other infected treated groups (Figures 5 and 6). The mean AST did not show any significant (P>0.05) difference in the infected treated groups. The mean ASP of infected untreated and DA groups were significantly (P<0.05) higher than the other infected treated group, however the resveratrol and DA treated group was significantly (P<0.05) lower than the resveratrol treated group and comparable with the control (Figure 7). The mean creatinine and BUN levels were significantly (P<0.05) higher in the infected untreated group. The creatinine level of the infected treated with both resveratrol and DA was significantly (P<0.05) lower than other infected treated groups, whereas the BUN did not differ significantly (P>0.05) within the infected treated groups when compared with the control (Figures 8 and 9). The mean albumin and total protein were significantly (P<0.05) higher in the infected untreated group, but the infected treated groups did not show any significant (P>0.05) difference However, the total protein was significantly (P<0.05) higher in the infected treated with resveratrol than in other infected groups (Figures 10 and 11).

Table 1: Mean (±SE) parasitemia (log10 trypanosomes/ml) of resveratrol supplemented *T. brucei* infected dogs treated with DA

Days post infection	Groups			
	Untreated	DA treated	Resveratrol treated	Resveratrol + DA treated
5	7.05±0.87	7.20±0.21		
6	7.65±0.20	7.50±0.17		
7	7.80±0.12	7.80±0.12		
8	7.86±0.14	7.80±0.12	7.20±0.12	7.20±0.12
9	8.03±0.14*	8.08±0.75*	7.65±0.87**	7.56±0.75**
10	8.40±0.12*	8.40±0.17*	7.9±0.75**	7.9±0.14**

Means ±SEM marked * is significantly different from mean marked **

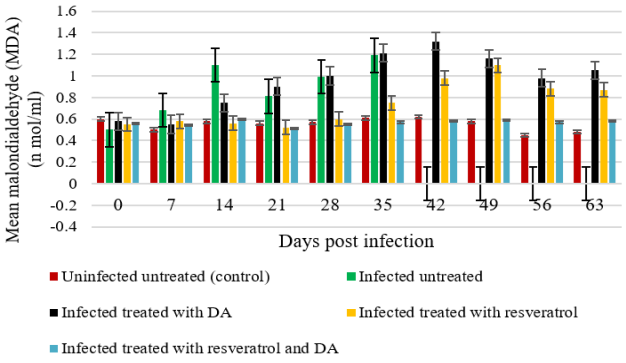

Figure 1: The mean Malondialdehyde (MDA) (n mol/ml) of resveratrol supplemented *T. brucei* infected dogs treated with DA.

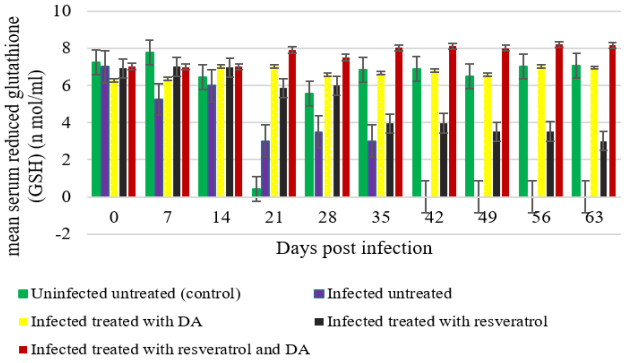

Figure 2: The mean serum reduced glutathione (GSH) (n mol/ml) of resveratrol supplemented *T. brucei* infected dogs treated with DA.

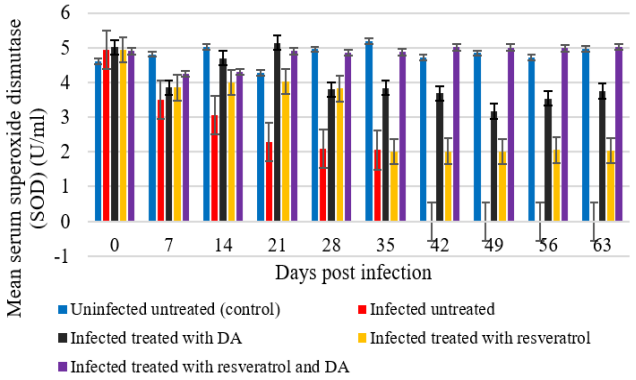

Figure 3: The mean serum superoxide dismutase (SOD) (U/ml) of resveratrol supplemented *T. brucei* infected dogs treated with DA.

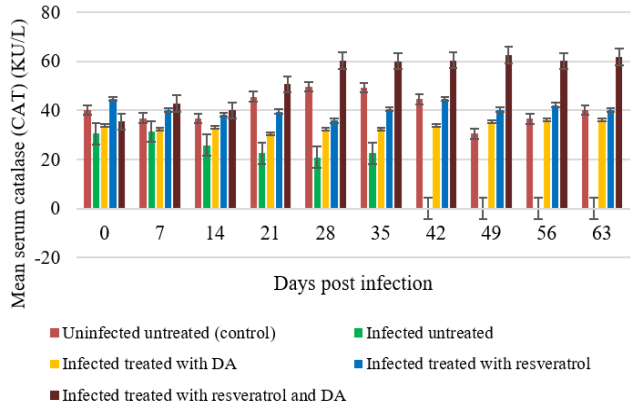

Figure 4: The Mean serum catalase (CAT) (KU/L) of resveratrol supplemented *T. brucei* infected dogs treated with DA.

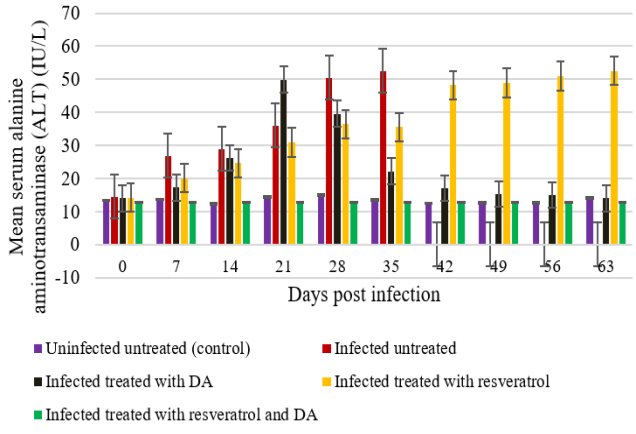

Figure 5: The Mean serum alanine aminotransaminase (ALT) (IU/L) of resveratrol supplemented *T. brucei* infected dogs treated with DA.

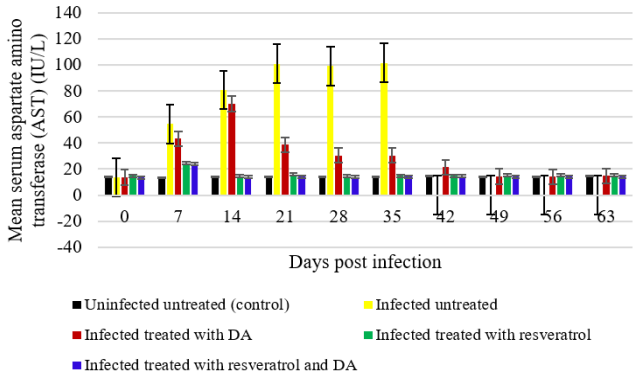

Figure 6: The Mean serum aspartate aminotransferase (AST) (IU/L) of resveratrol supplemented *T. brucei* infected dogs treated with DA.

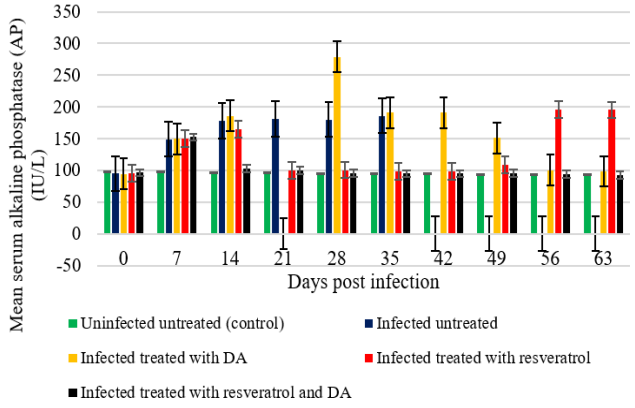

Figure 7: The Mean serum alkaline phosphatase (AP) (IU/L) of resveratrol supplemented *T. brucei* infected dogs treated with DA.

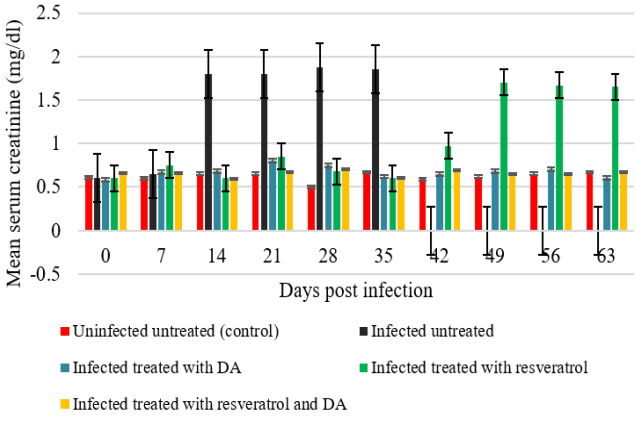

Figure 8: The Mean serum creatinine (mg/dl) of resveratrol supplemented *T. brucei* infected dogs treated with DA.

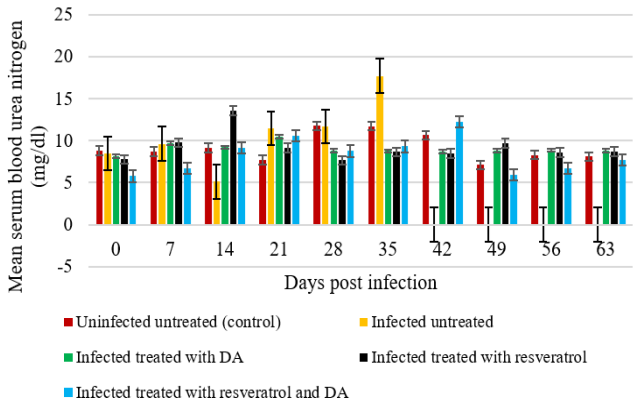

Figure 9: The Mean serum blood urea nitrogen (mg/dl) of resveratrol supplemented *T. brucei* infected dogs treated with DA.

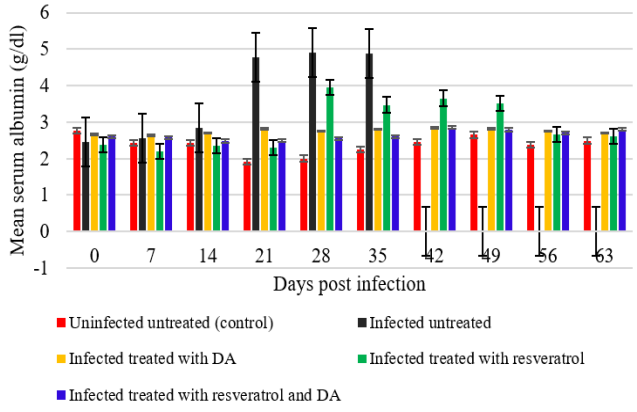

Figure 10: The Mean serum albumin (g/dl) of resveratrol supplemented *T. brucei* infected dogs treated with DA.

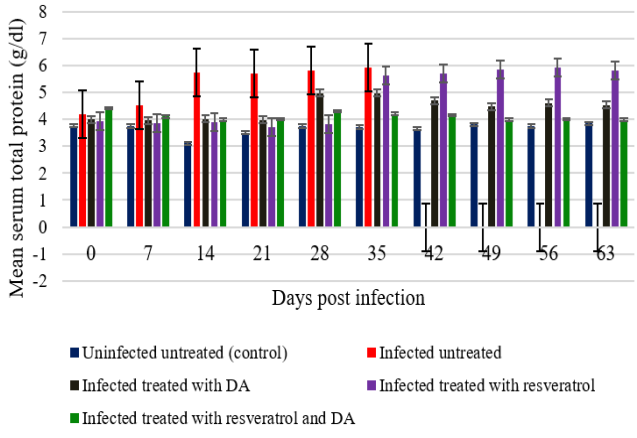

Figure 11: The Mean serum total protein (g/dl) of resveratrol supplemented *T. brucei* infected dogs treated with DA.

Discussion

Several studies have continued to report the promising effects of antioxidants supplementation in combating the scourge of African animal trypanosomosis (15,14), though antioxidants alone are ineffective in eliminating the trypanosome parasites from the blood of an infected animal (14). In this study, the parasitemia was first observed in the unsupplemented infected group with a mean pre-patent period of 5.75±0.96 in 5-7 days and increased progressively in the untreated group until deaths of the animals. The supplemented infected group had more extended pre-patent period of 9.00±0.82 in 8-10 days. The result shows that antioxidants delayed the onset of parasitemia in the supplemented groups. This result agrees with previous workers Eze and Ochike (15) and Eze *et al.* (16), who stated that antioxidants supplementation suppresses the occurrence of parasitema in infected animals. The findings could also be attributed to the immunomodulatory and antioxidants effects of resveratrol (25,26), which minimized the physiological stress, enhancing the immune response and antioxidant status of the infected animal (27). The significantly increased (MDA) observed in the infected untreated group indicates African animal trypanosomosis is associated with oxidative stress (15,28-30). The increase in the mean MDA is attributable to elicited increased production of pro-oxidants reported in trypanosomosis (31). The enhanced antioxidant GSH, SOD and CAT activities observed in the resveratrol and DA treated groups show that resveratrol is a powerful antioxidant and immune modulator (17,18). Immunosuppression is a significant manifestation of infection with trypanosome parasites (16). Several workers have reported increased MDA and decrease antioxidants markers in African trypanosomosis (16,32-34). The estimation activities of antioxidant markers are indirect ways to access the status of antioxidant defense in the body (34). Also, the decrease in the antioxidant activities post-infection could be attributed to the fact that infection with *T. brucei* leads to systemic depletion of antioxidants and decreased liver carotenoid concentration (31).

Infection caused a significantly increased mean serum ALT AST and ALP in the infected unsupplemented animals. Similar results have also been reported by previous workers Umar *et al.* (28), Takeet and Fagbemi (35), Yusuf *et al.* (36), and Hussain *et al.* (37), and are indicative of liver, heart and brain damage associated with trypanosomosis. The mean serum creatinine was significantly lower in the DA treated and both resveratrol and DA treated groups when compared with the control. The result agrees with Awobode's (38) findings, who reported significantly higher plasma creatinine levels in the *T. gambiense* infected population than in their uninfected control group. Abenga and Anosa (39) also observed a similar result in vervet monkeys infected with *T.*

gambiense. An elevated mean serum BUN was observed in the untreated, suggesting renal pathology. This result corroborates Abenga *et al.* (40), who observed an increased BUN in pigs infected with *T. brucei*. Similarly, elevated BUN levels by 50% have also been observed in red Sokoto goats infected with *T. vivax* (40).

A significantly higher mean total serum protein and albumin were observed in the infected untreated. The result agrees with the previous works of Abenga *et al.* (40), who observed elevated total protein in pigs infected with *T. vivax.* Similar results have been observed in humans, goats, rabbits, and monkeys infected with *T. rhodesiense* (40), *T. brucei* (41,42), respectively, and pigs infected with *T. brucei* (43). An increase total protein in African trypanosomosis is mainly due to hyperglobulinaemia and hypergammaglobulinaemia. It has also been associated with increased immunoglobulin M and dehydration, which is a consistent finding in trypanosomosis of man and animals (43). The hypoalbuminaemia was observed in the infected untreated, contrary to the results of other workers, Adejinmi and Akinboade (41), Sivajothi *et al.* (43), and Abenga and Anosa (39), who reported elevated albumin levels in trypanosome infected animals. However, variations in serum biochemical changes in trypanosomosis have been reported and are associated with different infecting species of trypanosomes and animal hosts (43).

From the results of this study, pretreatment with resveratrol before giving DA showed a protective effect against oxidative stress and reversed the damages done by the infection to the liver and kidneys. The superiority of resveratrol and DA combination over resveratrol and DA is mainly due to the protective and antioxidants effects of resveratrol leading to enhanced immune response (25,26). Several authors have reported the beneficial effects of antioxidants and micronutrients in trypanosomosis management. Improving host nutrition, through supplementation of antioxidants and micronutrients is essential in modulating the severity and pathophysiology of trypanosomosis and recovery (16). This is also in agreement with Akpa *et al.* (44), who reported the ability of diminazene aceturate to reverse the damages inflicted to the kidneys and liver in canine trypanosomosis. Pretreatment with resveratrol is beneficial in managing *T. brucei* infection, as it delays the onset of parasitemia and when combined with DA, it reverses damages done to the liver and kidneys.

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التأثيرات المفيدة للمكمل الريزفيراتول على الطفيلية والاجهاد التأكسدي و بعض المعايير الكيميائية الحيوية في الكلاب المصابة بداء المثقبات

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الخلاصة

هدفت هذه الدراسة الى التحري عن تأثير المكمل الريزفيراتول على الطفيلية والاجهاد التأكسدي وبعض المعايير الكيميائية الحيوية في الكلاب المصابة بطفيلي المثقبات من نوع Trypanosoma brucei. وكان معدل الفترة الزمنية لما قبل ظهور الاصابة الطفيلية (MPP) هو ٥,٧٥±٠,٩٦ (٥-٧) ايام، مقارنة مع الاصابة بدون اضافة المكمل واضافة المكمل ٩±٠,٨٢ (٨-١٠) يوم على التوالي، والتي أظهرت وجود فروقات معنوية (P<0.05). أما فعالية المالونداليديهايد (MDA) فاظهرت زيادة معنوية (P<0.05) في المجموعة المصابة مقارنة مع مجموعة المعاملة ومجموعة السيطرة. في حين وجد أن مستويات كل من انزيم ناقل الامين الاسبارتيت (AST) وانزيم ناقل الامين الالانين (ALT) في مصل الدم كانت اعلى من المجموعة المصابة المعاملة. بينما كان مستوى ALT في المجموعة المعاملة بالمكمل الريزفيراتول وعقار الديمينازين اسيجوريت AD منخفض معنوياً (P<0.05) عن مجموعة الاصابة. من جانب اخر كان مستوى كل من الكرياتينين ونتروجين يوريا الدم BUN مرتفع معنوياً (P<0.05) في المجموعة المعاملة المصابة. مستوى الكرياتينين في المجموعة المصابة المعاملة بالريزفيراتول و

AD كانت منخفضة معنوياً مقارنة مع باقي المجاميع المصابة ، بينما لم يتغير BUN معنوياً في المجاميع المعاملة مقارنة مع مجموعة السيطرة. أما مستوى الالبومين والبروتين الكلي فكان مرتفع معنوياً ($P<0.05$) في المجموعة المصابة غير المعاملة، بينما لم تظهر المجموعة المصابة المعاملة اي تغيير معنوي، في حين كان مستوى البروتين الكلي مرتفع معنوياً في المجموعة المصابة المعاملة بالريزفيراتول مقارنة مع باقي المجاميع. نستنتج من الدراسة الحالية ان العلاج القبلي بالريزفيراتول مفيد في علاج الاصابة بالمثقبات T.brucei، اذ انها تؤخر من بدء طفيليمية الدم وتعمل على اصلاح الاذى في الكبد والكلى عندما تعطى مع عقارAD.