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BIOCHEMICAL AND IMMUNOLOGICAL ASSESSMENT OF ANTITRYPANOSOMAL EFFECT OF NIGELLA SATIVA IN WEST AFRICAN DWARF BUCKS INOCULATED WITH *TRYPANOSOMA BRUCE*I

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ABSTRACT

African trypanosomosis remains a tropical disease with effects on livestock productivity and agricultural economies. Trypanosoma-brucei infection triggers prompt antigenic variation, and immunomodulatory responses. This study aimed at biochemical and immunological assessment of the antitrypanosomal effects of Nigella-sativa oil on T.brucei-West-African-Dwarf- (WAD) bucks. Sixteen (16) relatively healthy WAD bucks (11.4–12.4 kg, were randomised into four groups (n = 4): uninfected-control; infected + Nigella-sativa oil (40 µL/kg IV on days 22 & 26); infected + diminazene aceturate ; and infected-untreated. Infection was established by jugular-inoculation of 1 mL suspension containing 2×108 T. brucei/ml. Parasitaemia was monitored weekly. Serum samples at peak infection (day 21) and post-treatment (day 35) were assayed for catalase (CAT), superoxide-dismutase (SOD), reduced glutathione (GSH), malondialdehyde (MDA), interleukin-6 (IL-6), interleukin-10 (IL-10), and cardiac-troponin I (cTnI). Nigella-sativa oil decreased peak parasitaemia from 3.2 × 108 to 1.2 × 107 trypanosomes/ml, attaining 50 % survival; diminazene-aceturate cleared parasites by week-5 with 75 % survival. The disease caused marked decrease in CAT, SOD, and GSH with increased MDA, IL-6, IL-10, and cTnI ($p < 0.05$). Both treatments lowered MDA and raised CAT and SOD ($p < 0.05$), but only diminazene restored GSH. Nigella-sativa failed to normalise cytokines or cTnI, whereas diminazene returned IL-6, IL-10, and cTnI toward baseline. Intravenous Nigella-sativa oil shows mild antitrypanosomal effect and partial antioxidant enhancement, but cannot fully clear infection or stop myocardial injury. If integrated with diminazene-aceturate, Nigella-sativa might aid as supportive therapy to facilitate parasite clearance and alleviate oxidative and inflammatory damage.

Keywords: Nigella sativa, *T. brucei*, Cardiac injury, Oxidative stress, Cytokines, West African bucks

INTRODUCTION

African trypanosomiasis remains a major health problem to both humans and animals due to lack of effective treatment or vaccine to control the disease, it's a neglected zoonosis. The complex of diseases posed a threat to the livestock and agricultural subsection. Tesfaye *et al*.,2012 reported that about 3 million cattle die of trypanosomosis yearly with an estimated 3-5 billion dollars in lost annually. Also, the high cost and low availability of anti-trypanosomal drugs coupled with antigenic and drug resistance developed by the protozoan parasite (Weir *et al*., 2016). Tissue invasive nature of *T. brucei* could affect the heart, resulting in cardiac dysfunction (Caljon *et al*., 2016). Cardiac dysfunction in relation to trypanosomosis has been linked to excess production of reactive oxygen species (ROS) at the cellular level due to oxidative stress (Machado-Siva *et al*.,2016). Trypanosomes multiply rapidly after invading mammalian body to form a population within the infected host (Shukla *et al*., 2024). The ability of the parasite to develop a wide repertoire of antigens renders the host's antibodies against the parasite ineffective. In terms of antigen-antibody interaction, the host immune system is not perfectly specific and regularly lags behind the course of the disease (Sternberg, 2004). The host immune system eventually collapses and the parasites infiltrate the central nervous system, resulting in a coma and eventual death.

Trypanosome infection can result to a remarkably increase in the production of reactive oxygen species (ROS), which function as cytotoxic agents and are require in the pathophysiological mechanism of trypanosomosis (Paiva et al., 2018). According to Abubakar and Dabo, (2023) evaluating the performance of oxidative stress indicators in trypanosomosis can elucidate on the inflammatory response of the host and the cellular damage caused by the parasite, allowing for the development of efficient management and treatment plans that mitigate the impact of this debilitation disease. Particularly with therapies like isometamidium and diminazene, trypanocidal drug resistance is an increasing concern. The work of Okello et at. (2022) revealed that *T. congolense* possessed the most resistance rate due to anaemia According to the work of Okello et al. (2022), *T. congolense* has the most resistance rates, ranging from 11% to 83%. Owing to anaemia, loss of condition, and reproductive problems, African animal trypanosomosis leading to a large financial losse for animals. According to Yaro et al. (2016), the disease is a vital hindrance to the production of cattle, with losses approximated to be as high as $4.5 billion annually. Different cytokines are concerned in mediating the immune response to trypanosome infections. There is a link between the host's ability to lessen parasitaemia and pathology and the change in cytokine production from pro-inflammatory to anti-inflammatory during late or chronic infection (Baral, 2010). In mouse models, Namangala et al. (2001) found that the balance between pro- and anti-inflammatory cytokines is crucial for the disease course. Early on in an infection, interleukin 12 (IL-12), nitric oxide (NO), interferon-gamma (IFN-γ), and tumour necrosis factor alpha (TNF-α) are produced; later, interleukin 13 (IL-13), interleukin 4 (IL-4)

and interleukin 10 (IL-10) are produced (Baral, 2010; Tylor and Mertens, 1999; Uzonna et al., 1999). Human African trypanosomiasis in its late stages has been demonstrated to have increased levels of IL-10 and IL-6 (Kato et al., 2015). Cytokines have been suggested as prospective diagnostic or stage biomarkers due to the overexpression of various cytokines at different phases of infections (Kato et al.,2016). Despite being used for over 50 years, diminazene, isometamidium, and homidium are linked to extreme toxicity and parasite resistance. The threat of the disease has not been eliminated despite numerous laboratories attempts over the years to develop novel treatment profiles through combination therapy, pharmacokinetic investigations of trypanocides, use of antioxidants, and usage of medicinal herbs. The antigenic diversity of the trypanosome surface coat, which has hindered or even completely stopped vaccine development, has also contributed to the failure of efforts to develop an effective vaccine (Eghianruwa and Oridupa, 2018). Due to the aforementioned reasons and implications this study is aimed at assessing the antitrypanosomal effect of *Nigella sativa* on the efficacy of its immunological and biochemical properties.

MATERIALS AND METHODS

Drug Application

Diminazene aceturate (survidim® manufactured by LAPROVET, France) was administered at a dosage of 3.5mg/kg was administered via intramuscular route to bucks in group C on day 22

Nigella sativa (black seed oil) manufactured by HEMANI®, Pakistan was administered at 40µL/kg intravenously using the method of El-Tahir *et al*., (1993); Akbar, (2018) on day 22 and 26 respectively to bucks in group B. A sample of the black seed oil was incubated for 72 hours for the growth of any pathogens and it was pathogen free before administration.

Ethical Approval

This study was conducted at the Department of Veterinary Medicine, Faculty of Veterinary Medicine, University of Ilorin. Ethical approval for this study was sought from University Research and Ethical Committee, University of Ilorin, Nigeria with approval code number UREC/FVM/14/32TA068/2023. All ethical guidelines regarding the treatment, care, and welfare of the animals were carefully adhered to during the entire study, from its beginning to its end.

Experimental Animals and Study Design

Sixteen adult healthy WAD bucks were purposefully used for this trial. The bucks weighing between 11.4-12.4 kg. The goats were housed in the goat pen at the University of Ilorin Veterinary Teaching Hospital. The universal body score condition of the experimental animals was between 3 and 3.5 on a scale of 5. The animals were acclimatized for 2 weeks, during which they were screened initially and found negative for trypanosomes using the HCT and Buffy coat methods (Woo, 1971); (Murray et al., 1977. They were fed on beans husks, wheat shafts, dried cassava peels, grass and water was given *ad libitum.* The experimental animals were randomly allotted into four groups of four animals each.

The experimental dosages were as follows: Group A: Negative control and the goats were uninfected and untreated. Group B: Infected and doses with *Nigella sativa* at day 22 and 26 respectively at 40µL/kg slow intravenously following the method of EL-Tahir et al., 1993; Akbar (2018). Group C: Infected and treated with diminazene aceturate at 3.5 mg/kg intramuscularly. Group D: Positive control, infected and not treated.

Trypanosoma Stock and Inoculation

Trypanosoma brucei (*Federe* strain) was source from the Veterinary and livestock studies Department, National Institute for Trypanosomiasis and Onchocerciasis Research Institute, Vom, Nigeria was used in this study. The parasite was maintained by serial passage in rats not included in the present study by pricking the tail to obtained blood. The blood was mixed with 0.9% normal saline and 0.5 ml of the suspension was administered through the intraperitoneal route using the method of Leigh *et al* (2015) in the rats. Parasitaemia was monitored and detected by placing a drop of blood from the tail vein of the infected rats on glass slide, after which a cover slip was place on it and viewed under ×100 magnification.

Blood was obtained from rats with high levels of parasitaemia to prepare parasite suspension in 0.9% normal saline (1:1) according to the methods of Leigh *et al* (2015). 1ml of the suspension was injected into the jugular vein of the goats in groups B, C and D after aseptically preparing the site using a 21G needle. The dose of inoculum was $2x10^8$ (8.3 antilog) Parasite of *T. brucei* per ml of blood using the Rapid Matching method described by Herbert and Lumsden (1976).

Parasitaemia Assessment

10 days post inoculation with parasite suspension, blood samples were collected using a 5ml syringe and a 21G needle from the jugular veins of all the buck, the site was disinfected with Isopropyl Alcohol (MOKO®). The parasitaemia level was monitored using wet mount method as described by Abdulfatai *et al* 2017, and the result was recorded. Blood sample was collected pre and post inoculation for analysis.

Biochemical Analysis

Blood sample was collected at the peak of parasitaemia that is day 21 and after treatment on day 35 from the goat via the jugular vein was left for 2 minutes at room temperature before centrifugation for 20 minutes at 1000 revolution. The serum obtained was analyzed using commercial test kits Elabscience®, USA to assay for cytokine such as interleukin-6 (IL-6) with ELISA Kit Catalog No: E-EL-R0015 96T, and interleukin-10(IL-10) with ELISA Kit Catalog No: E-EL-R0016 96T, cardiac troponin I- with ELISA Kit Catalog No: E-EL-R1253 Product size: 96T/48T/24T/96T*5 TNNI3/cTn-I (Troponin I Type 3, Cardiac), antioxidants such as superoxide dismutase activity using the pyrogallol method, catalase activity using the molybdate method, reduced glutathione (gsh) colorimetric method, magnesium ions activity using the xylidyl blue method and oxidative stress marker such as malondialdehyde (mda) concentration. All absorbances were determined using spectramax (340) microplate reader.

Data Analysis

All data generated were expressed as Mean ± Standard deviation. The difference between the groups were analyzed by one-way analysis of variance (ANOVA) followed by Tukey's posy-hoc multiple comparison test using Graphpad Prism 10.03 statistical package, San Diego, California, U S A (www.Graphpad.com). Values of parameters were compared and the level of significance was set at $P \leq 0.05$.

RESULTS AND DISCUSSION

Parasitaemia

The parasitaemia level was determined as described by Ameen et al.,(2008); Daba and Maigari, 2018). The bucks in group A depict no parasitaemia across the groups, indicating no of infection. Group B exhibited a peak parasitaemia of 8.6 (antilog ≈ 3.2×10^8 trypanosomes/ml) which declined to 7.1 (≈ 1.2×10^7) by week 5 post treatment with Nigella sativa. Group C reached a peak of 8.7 (≈ 3.9×10^8) by week 3 and achieved complete parasite clearance (0.0) by week 5 with treatment of diminazene aceturate. Group D showed a steady increase in parasitaemia, reaching a 9.0 (≈ 1×10^9) by week 5, showing progressive infection in absence of treatment as shown on the figure 1

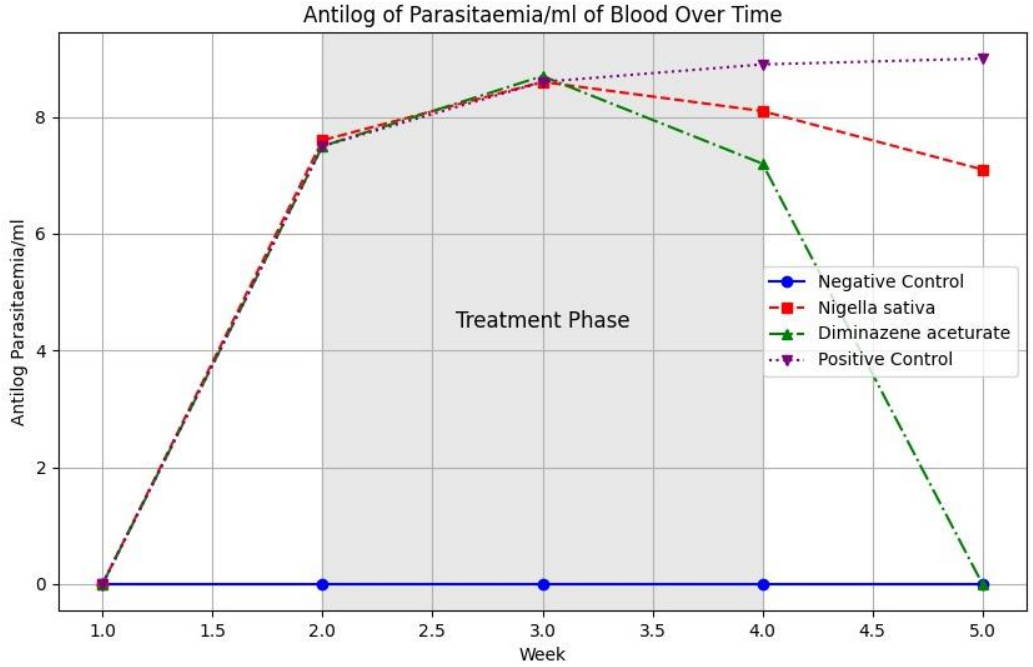

Figure 1: Antilog of parasitaemia/ml of blood over 5 weeks in West African Dwarf bucks. Group A (negative control), stayed clean. Group B was inoculated with the parasite and was treated with Nigella sativa, showing a small decline in parasitaemia. Group C was infected and treated with Diminazene aceturate, signifying a swift clearance by Week 5. The positive control group(D), reveals a continuous rise in the parasitiaemia level, given that no treatment was administered. The shaded region indicates the treatment phase (Weeks 2–4).

Antioxidant Enzyme Activity

Investigation from the biochemical profile revealed a significant decreased ($p<0.05$) in the antioxidant and oxidative stress markers among the groups. Infection with T. brucei demonstrated a significant oxidative impairment. A dropped in the levels of glutathione, superoxide dismutase and catalase demonstrated this impairment. In the same vein, the was an elevated level of malondialdehyde, which signified lipid peroxidation.

Catalase Activity (CAT)

CAT activity was significantly decreased (18.0 ± 5.15 µ/ml, $p < 0.05$) in group B during infection, but was restored after treatment with Nigella sativa (23.4±5.41) in comparison to the control (26.70± 2.94µ/ml). Diminazene aceturate did not improve catalase activity in group C (20.50±7.35µ/ml). The positive control showed a significant decrease (19.0±0.92 µ/ml, $p < 0.05$) compared to the negative control (26.70±2.94 µ/ml).

Superoxide Dismutase Activity (SOD)

SOD level was greatly reduced significantly ($p<0.05$) in all infected groups with group C during infection (0.3±0.19 µ/ml) and bucks in group D (0.31±0.22 µ/ml) in comparison with the control (2.53±1.76 µ/ml). After treatment, bucks in both group B and C shown some recovery of SOD. With those in group C at 1.31±1.28 µ/ml surpassed those in group B which was at 1.16±0.65 µ/ml.

Glutathione Activity (GSH)

There was no significant difference ($p > 0.05$) in either the bucks during infection and treatment with intravenous Nigella sativa oil or those treated with diminazene produced substantial changes in systemic GSH levels in comparison to the uninfected control under the conditions tested.

Magnesium Ions (Mg^{2+})

Magnesium level was steady in all the groups, and there were no significant differences ($p> 0.05$) between the control and the rest of the groups. Magnesium ion highest concentration was recorded in the Nigella sativa group at 1.80±0.54 mmol/L when compared to the Diminazene aceturate group, 1.54±0.09. Group D recorded the lowest Mg2+ concentration, 1.23±0.29.

Oxidative Stress Marker

Malondialdehyde (MDA)

During infection, the concentration of MDA was elevated in all the infected groups, with the highest concentration in group C (4.29±0.81) and group B (4.19±1.25) during the course of the infection, when compared to the control (3.03±1.75). However, after treatment with Nigella sativa and Diminazene aceturare, there was a slight reduction in MDA in group B (3.53±0.56) and C (3.31±0.85), respectively.

Table 1: Antioxidants, and Stress Marker Results (Mean ±SD) of WAD Bucks Infected with T. Brucei and Treated with Nigella Sativa and Diminazene Aceturate

Parameters	A(control)	B_I	B_T	C_I	C_T	D
CAT(µ/ml)	26.70±2.94[a]	18.5±5.15[b]	23.4±5.41[a]	20.20±0.19[a]	20.50±7.35[a]	19.0±0.92[c]
SOD(µ/ml)	2.53±1.76[a]	0.69±0.68[a]	1.16±0.65[a]	0.34±0.19[a]	1.31±1.28[a]	0.65±0.73[a]
GSH (mM)	1.08±0.49[a]	1.11±0.14[a]	0.75±0.15[a]	1.49±0.38[a]	0.87±0.31[a]	0.76±0.13[a]
Mag^{+2}(mmol/L)	1.45±0.33[a]	1.41±0.29[a]	1.80±0.54[a]	1.56±0.09[a]	1.38±0.04[a]	1.23±0.25[a]
MDA(µm/ml)	3.03±1.75[a]	4.19±1.25[a]	3.53±0.56[a]	4.29±0.81[a]	3.31±0.85[a]	4.17±1.36[a]

Superscripts of different letters ([a], [b], [c], [d],[e]) indicate statistical significance (p < 0.05) between groups.

Interleukin-6(IL-6) Pro-inflammatory Marker

There was a significant (p<0.05) increase in values of IL-6 in all the groups during infection. After treatment, the *Nigella sativa* group still shows a significant (3.53±0.58, p<0.05) increase in the values of the IL-6 whereas there was non-significant (2.90±0.86, p>0.05) decreased in the values of the IL-6 in the diminazene aceturate treated group in comparison to the control (1.48±0.23).

Iterleukin-10(IL-10) Anti- inflammatory Marker

During infection, all the infected groups shown a significant increased (p<0.05) in the level of interleukin-10 due to compensatory inflammatory response. After treatment, Nigella sativa did not normalize the bucks in group B as IL-10 was still significantly higher (1.61±0.15) where as those treated with diminazene aceturate (group C) shown a non-significantly decreased (1.46±0.34, p>0.05) from the control (0.79±0.12) hence moderate immunomodulatory effect. The infected and untreated group demonstrate a continuous increase of IL-10 reflecting persistence inflammation.

Cardiac Injury Marker: cTn I

There was a significant increase (p<0.05) in cardiac troponin I (cTn I) in all affected groups with bucks in the positive control group having the greatest value (67.19±3.30 pg/ml) when compared with negative control (38.42±11.16 pg/ml) during infection. After treatment, the Nigella sativa did not reduce cTn I as the value was significantly high (64.26±4.32, p<0.05) when compared to the bucks in group C that were treated with diminazene aceturate had value (56.32±3.44) that were statistically comparable to the negative control, indicating a better cardiac protection.

Table 2: Interleukin and Cardiac Troponin I Result (Mean ±SD) of WAD Bucks Infected with *T Brucei* Treated with *Nigella Sativa* and Diminazene Aceturate

Parameters	A(control)	B_I	B_T	C_I	C_T	D
IL-6(pg/ml)	1.48±0.23[a]	3.56±1.07[b]	3.77±1.7[bc]	3.16±0.46[bd]	2.90±0.86[ab]	3.53±0.53[bc]
IL-10(pg/ml)	0.79±0.12[a]	1.97±0.85[b]	1.59±0.15[bc]	1.67±0.22[bd]	1.46±0.34[a]	1.61±0.29[be]
cTn I (pg/ml)	38.42±11.16[a]	63.19±20.61[b]	64.26±4.32[bc]	60.83±12.78[a]	56.32±3.44[a]	67.19±3.30[bd]

Superscripts of different letters ([a], [b], [c],[d],[e]) indicate statistical significance (p < 0.05) between groups.

Discussion

Nigella sativa has a rich history of being recognised for its potential antioxidant properties. Research by Kazemi (2014) and Singh et al. (2014) suggests that it lowers the levels of reactive oxygen species (ROS) while enhancing antioxidant enzymes, such as superoxide dismutase, catalase, and glutathione. El-Gindy et al. observed a notable increase in total antioxidant capacity (TAC) in the blood and a decrease in malondialdehyde (MDA) in rabbits that received a supplement of 600 mg/kg of black seeds (El-Gindy et al., 2020). Additionally, Nigella sativa exhibits anti-inflammatory properties attributed to its active components, black cumin and thymoquinone (TQ) (Dwita et al., 2019). The oil extract of *Nigella sativa* has been shown to lower interleukin-6 levels in cases of low-grade inflammation in human pre-adipocytes (Bordoni et al., 2019). TQ is the primary compound that contributes to the anti-inflammatory effects of black cumin. Hossen et al. found that TQ reduced pro-inflammatory factors, including nitric oxide (NO), nitric oxide synthase, tumour necrosis factor, IL-6, IL-1, and cyclooxygenase-2, in lipopolysaccharide-activated murine macrophage-like RAW264.7 cells, involving a mechanism that encompasses the inhibition of IRAK-linked AP-1/NF-κB pathways (Hossen et al., 2017).

Trypanosomes multiply rapidly after invading the mammalian body to form a population within the infected host (Shukla et al., 2024). The parasite's ability to develop a wide repertoire of antigens renders the host's antibodies against it ineffective. In terms of antigen-antibody interaction, the host defence mechanism is only partially selective and frequently lags behind the course of the disease (Sternberg, 2004). The host immune system eventually collapses, and the parasites infiltrate the central nervous system, resulting in a coma and eventual death. Removing the parasite from the body while also enhancing the host's immune system could be crucial in managing African sleeping sickness (Hoet et al., 2004; Chibale, 2005).

The bucks in group B presented a significant decrease in parasitaemia after treatment with Nigella sativa, although not complete clearance. The decrease from 3.2×10^8 to 1.2×10^7 trypanosomes/ml and survivability of 2/4 bucks indicates that Nigella sativa holds mild antitrypanosomal properties. This is in confirmation with previous work of Abd-EL-Hakim et al (2021); Ekanem and Yusuf (2008) who reported a significant decreased in parasitaemia in sheep inoculated with *T. evansi* and rats inoculated *T. brucei* respectively when treated with NS. This may be due to its bioactive compound thymoquinone that may exert an antiparasitic effect through immunomodulation. In comparison with the bucks treated with diminazene aceturate, there was rise in parasitaemia at day 3 but it was cleared completely after treatment with 3/4 bucks survive. This is a strong evidence for the potent antitrypanosomal of diminazene aceturate. Therefore, this study showed that intravenous administration of Nigella sativa oil does not possess antitrypanosomal activities against T. brucei-infected West African dwarf bucks since it could only reduce the parasitaemia and not cleared it completely. Nigella sativa could serve as a complementary therapy or alternative in settings where conventional drugs are limited.

The positive control group of animals undergo a constant rise in parasitaemia throughout the study, attaining the peak by week 5, with just 1/4 of the bucks in this group surviving. This

validates the virulence of the inoculated trypanosome and corroborates the experimental model. The absence of unexplained recovery emphasises the importance of therapeutic intervention.

Infections caused by trypanosomes lead to the production of large quantities of free radicals and reactive oxygen species (ROS), which are harmful to cellular components like lipids and proteins which are essential for cell survival (Morrison et al.,2023). Furthermore, research has consistently shown that the oxidative stress induced by these free radicals is a significant contributor to the progression of trypanosomosis, as noted by Ogunsanmi and Taiwo in 2001 and Akanji *et al*., 2009.

The oxidative damage caused by T. brucei was monitored by serum activities of CAT, SOD, GSH, Mg^{2+}, and MDA. Adeyemi and Sulaiman (2012) state that measuring antioxidant enzyme activities such as SOD, CAT, and GSH is a suitable means of indirectly evaluating the level of antioxidant defense against trypanosomosis. The results of this study indicated non-significant ($p>0.05$) alteration in the antioxidants during infection due to the *T brucei* influenced which is in line with the works of Adeyemi and Sulaiman (2012). This was seen due to the non-significant ($p>0.05$) increased of the mean lipid peroxide in the form of malondialdehyde (MDA) which induces reactive oxygen species (ROS) causing oxidative stress on the host red blood cells and other organs (Pandey *et al*.,2015). This is in support with the works of Ranjithkumar *et al*. (2011); Wolkmer *et al*. (2009); Ogunsanmi and Taiwo (2001) who also report increase in lipid peroxidation as a result of oxidative stress induce by the parasite. After treatment, there was a non-significant ($p>0.05$) decrease in the value of MDA in both the Nigella sativa group and the diminazene aceturate group. This was due to the non-significant ($p>0.05$) increase in the values of CAT and SOD in both treatment groups. Nigella sativa improved the antioxidant status of the bucks which may be due to it active principles thymoquinone suggesting it potential as complementary therapy. This is in consonance with the work of Abd El-Hakim *et al*.,2021; Alzohairy *et al*., 2021 who also observed decrease in MDA and increase in antioxidant activities in sheep and rats respectively with the use of Nigella sativa. Diminazene showing superior efficacy in restoring antioxidant balance, particularly in enhancing SOD levels and reducing MDA concentrations more.

Nigella sativa did not restore the GSH level which is not in agreement with the work of Liu et al,. (2022) whose work with Nigella sativa active component thymoquinone increases the GSH levels and prevent oxidative damage in experimental autoimmune encephalomyelitis; Ekanem & Yusuf, (2008). This may be due to the route and formulation consequently the extract may not have distributed successfully to the liver, spleen or macrophages which are the site for T. brucei proliferation (Anosa & Kaneko, 1984). This may also be due to the bucks compensating to the other antioxidants (SOD and CAT) pathways. This also suggest that the GSH was consume during oxidative stress neutralization (Sultan et al., 2015).

The infected untreated bucks manifested a significant reduction in CAT, SOD, and GSH, with also raised MDA levels. These biochemical changes reveal that T. brucei can induce lipid peroxidation, hence overpowering the host antioxidant defence system through the production of high reactive oxygen species. This observation aligns with findings from Akpa et al. (2021), who also recorded similar oxidative stress markers in Nigerian local dogs experimentally infected with T. brucei, indicating a decrease in antioxidant enzyme activities and an increase in MDA levels. Additionally, Banwo et al. (2024) demonstrated that naturally infected bovines exhibit significant oxidative stress, characterised by increased MDA and decreased SOD and GSH levels. The findings further substantiate the widespread damage T. brucei causes to cellular structure and immune function, in agreement with similar work on oxidative profiles induced by trypanosomosis across species, emphasising the systemic impact of the parasite on cellular integrity and immune function.

Magnesium concentration showed no significant variation across all experimental groups, demonstrating that T. brucei and the therapeutic intervention with Nigella sativa or diminazene aceturate did not change magnesium homeostasis. This finding corroborates the observation of Nwoha et al. (2009), who also reported stable magnesium ion levels in WAD goats naturally infected with T. vivax and treated with diminazene aceturate. The inflammatory level provoked by T. brucei was observed by serum activities of cytokines (IL-6 and IL-10). The results of this study depicted a significant ($p < 0.05$) increase in the values of IL-6 and IL-10 in the T. brucei-infected WAD bucks, as observed when compared to the uninfected control group during the course of the infection. Post-treatment evaluation showed that bucks administered Nigella sativa demonstrated a persistent significant increase ($p < 0.05$) in cytokine levels. In opposition to the group treated with diminazene aceturate, it revealed a non-significant reduction ($p > 0.05$) in IL-6 and IL-10 concentration. The significant increase in the cytokine levels observed during the course of the T. brucei infection demonstrates an essential part of the host's immune response. According to Baral (2010), when the parasite invades the host, it triggers the activation of a Th1-type immune response, during which skin and natural killer cells release pro-inflammatory cytokines like IL-6. These cytokines stimulate pro-inflammatory macrophages, which exert a direct parasiticidal effect in the extravascular tissues, thereby prolonging inflammation and aiding in parasite control. Also, stimulation of B cells by macrophages and dendritic cells by the production of IL-6 and IL-12 eases a straight wave of parasite clearance in the bloodstream by targeting the variable surface glycoprotein of the T. brucei (Caljon et al., 2018). This immunological cascade results in the increase in IL-6 levels during the infection.

As the inflammatory process continues, Th2 releases anti-inflammatory cytokines (IL-10) that help with the downregulation of the pro-inflammatory process and immunosuppressive effects, which may minimise tissue damage and improve the survival of infected goats (Magez and Radwanska, 2009). The ability of the IL-10 to suppress the pro-inflammatory cell accounts for its elevated level. The results of this study indicated that *Nigella sativa* treatment did not dampen immune cell activation of pro-inflammatory cytokines (IL-6); this is evidenced by the significant ($p<0.05$) increase in its value. This is not in agreement with the finding of Koshak *et al*. (2018) and did not improve the anti-inflammatory activity of the active compound (thymoquinone) of *Nigella sativa.* This is in contrast to the finding of Majdalawieh *et al*. (2010) and Kato *et al*. (2016), whose finding revealed that *Nigella sativa* oil extract favours the secretion of Th2 that is responsible for humoral immune responses by suppressing pro-inflammatory mediators. The discrepancy between the current study and the previous study could be due to dosage and duration of the Nigella sativa oil that may may be insufficient to produce large anti-inflammatory response, the species of animal model use and probably the severity of inflammation caused by the parasite may have overshadow the modulatory effects of IL-10. In comparison with the group that was treated with diminazene aceturate, the drugs did clear the parasite and markedly

modulated the host immune response. This was seen as the serum level of IL-6 and IL-10 was non-significantly reduced. Thus, the drugs restore immunological balance by dampening excessive inflammatory and anti-inflammatory signals (Kuriakose et al., 2012). This finding is in line with the previous work of Kuriakose et al. (2012), who show that diminazene aceturate treatment in T. congolense-infected mice had a marked reduction in serum levels of IL-6, IL-12, and IFN-γ, with a drop in regulatory T cell population ($CD4^+$ $FoxP3^+$)

The reThe myocardial damage caused by trypanosome was monitored by serum activities of cardiac troponin I (cTnI). sults of this study indicated a significant ($p>0.05$) increased in cTnI level in all the groups during infection which corroborate with the gravity of parasitaemia and immune response resulting to myocardial injury. This is in line with the work of Crilly et al. (2025) in cattle infected with T. brucei who noticed increased cTnI, which was connected to associated with high levels of inflammatory cytokines like IFN-γ and IL-10, indicating an immune-mediated unit of cardiac injury. The increase in cTnI level could be also due to the invading parasite into the heart tissue that will cause oxidative stress and this will increase the level of the lipid peroxidation and inflammation brought on by the parasite (Pandey *et al*.,2015). The group treated with *Nigella sativa* did not respond favourably with the treatment as this was observed with an elevated serum cardiac troponin level. This could also be seen as there was no significant decrease of the pro-inflammatory cytokines. Thus, Nigella sativa did not ameliorate the anti-inflammatory action against *Trypanosoma brucei* in the heart. This finding did not correlate with the work of Norouzi *et al*., (2017) whose finding suggest that *Nigella sativa* significantly reduced the pro-inflammatory markers and improved the anti-inflammatory markers in rats. This inconsistency could be due the dosage, and animal model used. The elevated cTnI level in the goats treated with diminazene aceturate decreased non-significantly ($P>0.05$) but the concentration was above the control. This is in line with the work of Abakpa *et al*., (2020) who found that dogs treated with diminazene aceturate had increased cardiac troponin I level following *T congolense* inoculation. This indicates that the goats were not fully recovered from the infection. This may also imply that cTnI serum level in myocardial injury as result of *Trypanosoma brucei* infection does not promptly revert to normal level following therapy.

CONCLUSION

Intravenous Nigella sativa oil reduced parasitaemia and produced a 50% survival rate, demonstrating a mild antitrypanosomal effect, in contrast, diminazene aceturate cleared parasites, and achieved a75% survival rate. The parasite induced oxidative stress identified by drop in CAT, SOD, and GSH, and increased MDA. Both treatments lowered MDA and increased CAT and SOD, but only diminazene fully improved antioxidant balance; thymoquinone raised CAT and SOD but did not recover GSH. Infection elevated IL-6 and IL-10; Nigella sativa did not reduce these cytokines, whereas diminazene returned them toward baseline. Infection-induced myocardial injury with increased cTnI; Nigella provided no cardiac protection, and diminazene produced only a modest, non-significant cTnI decline. Nigella sativa can be used as an ameliorative supplement to reduce the level of parasitaemic pattern and prompt clearance of the parasites when combined with potent conventional trypanocides such as diminazene aceturate.

This research is not devoid of its constraints but the findings suggest a promising therapeutic potential. In upcoming studies, we intend to systematically investigate how this combination can be synergistic and optimal for therapeutic windows.

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