Effects of levamisole on haematological and oxidative stress parameters in packed donkeys

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Abstract

Stress can be caused by psychological, physiological, environmental and physical factors. Strenuous exercise like packing in donkeys modifies haematologic parameters. The aim of the study was to investigate the ameliorative effects of levamisole on stress, in packed donkeys. 15 adult male donkeys aged between 4 – 5 years were selected for this study, divided into groups (A, B, C) of five donkeys each: Groups A and B donkeys were the apparently healthy group; while Group C donkeys were naturally infected with Strongyle spp. All the donkeys participated in load carrying (packing) of 40 kg for 10 km. Groups B and C were treated with levamisole at 7.5 mg/kg, while Group A received no treatment prior to packing. Blood was collected from all the groups for haemogram and oxidative stress biomarker analyses. No significant effect ($P > 0.05$) was observed between groups: A, B and C for: respiratory rate, pulse rate and rectal temperature; haemogram, and activities of malondialdehyde concentration, superoxide dismutase and catalase. Erythrocyte membranes were osmotically stable at 0.5% NaCl in the treated group in comparison to the controls. Packing of donkeys for 10 km did not induce significant changes in vital parameters, haemogram and biomarkers of oxidative stress, but levamisole improved erythrocyte membrane stability. It was concluded that packing for 10 km did not induce any significant changes in blood cellular components and biomarker of oxidative stress, but levamisole improved erythrocyte membrane stability.

Introduction

Common pack animals used throughout Africa and the Middle East include donkeys, mules, oxen, and dromedary camels (Amber, 2017). Donkeys (*Equinus asinus*) are hardy, docile, intelligent and animals that can be easily trained for load carrying activity (packing) in many rural small-holder communities (Bale et al. 2003; Minka and Ayo, 2007). Most donkeys are reared and managed extensively. Historically, donkeys are well known for their use, especially in transportation, and cultivation of land. (Fernando and Starkey, 2004; Hanekom, 2004). In India, donkeys are used mainly as pack animals for transporting bricks and goods (Pal and Gupta, 2002). Pack donkeys are used to carry or transport goods or supplies (Loescher, 2015). Donkeys are preferred to other equines because of their affordability, survivability, docile nature and ease of training and handling (Swai and Bwanga, 2008). Strenuous exercise in donkey cause changes in haematological parameters (Olaifa et al. 2015). Packing in donkeys is a form of physical exertion that may be stressful. The sympathetic nervous system plays an important role in the mediation of responses to exercise by modifying cardiovascular function resulting in alterations of haematologic parameters (Olaifa et al. 2015). Oxidative stress results from an imbalance between radical generation occurring during normal homeostatic mechanisms in the body, when oxygen is metabolised or burned, and radical-scavenging (antioxidant activity) activities resulting from increased production of pro-oxidants and / or a decrease in antioxidant defence (Bernabucci et al. 2002). Over production of reactive oxygen species (ROS): superoxide anion (O2 -), hydrogen peroxide (H_2O_2), hydroxyl radical (OH.) disrupts the balance between the pro-oxidant / anti-oxidant system (Aluwong et al. 2013). Whenever the disruption favours the pro-oxidants, oxidative stress usually occurs. The ROS are oxidants generated during normal

metabolism or homeostasis within a host's body, they make up about 1-2% of the body and are involved in functions, including protein phosphorylation, cell maturation, apoptosis, oocyte maturation and steroidogenesis. However, their levels increase during certain pathological conditions, resulting in oxidative damage of biological macromolecules and disrupt normal functions (Celi et al. 2012). Packing in donkeys is a form of stress and ultimately leads to an imbalance between pro-oxidants and anti-oxidants (Pearson and Vall, 1998; Minka and Ayo 2007; Olaifa et al. 2015). Packing has been shown to increase the concentration of Malondialdehyde concentration and activities of superoxide dismutase and catalase. It has been demonstrated the disease-modifying antirheumatic property of levamisole, which has been useful in the treatment of rheumatoid arthritis (Olsen et al. 1988; Sany, 1990; Popovic et al. 1998). levamisole acts as an immunomodulator and immuno-enhancer by increasing macrophage chemotaxis and T-cell lymphocyte function. It stimulates neutrophil chemotaxis, up-regulate toll-like receptors, and enhance dendritic cell maturation (Lee et al. 2012). It inhibits lipid peroxidation, and known to have radio-protectant properties which has been demonstrated in anti-cancer activity, when combined with fluorouracil (5-FU) as adjunct treatment for colon carcinoma (Artwohl et al. 2000). Different studies have shown the antioxidant properties of levamisole, in particular, about its effects on the major cellular redox system glutathione (GSH/GSSG) (Ince et al. 2010; Lake et al. 2012).

Materials And Methods

Ethical clearance

Ethical clearance was obtained before the commencement of the experiment from the Animal Use and Welfare Committee of the Ahmadu Bello University, Zaria, Nigeria with reference number ABUCAUC/2019/25

Study area

The study was conducted in the National Animal Production Research Institute (NAPRI), Shika – Zaria, Nigeria located, in the Northern Guinea Savanna zone of Nigeria with latitude 11º 12/N, longitude 7º 33/E and an altitude of 610 m above the sea level (Mohammed et al. 2007).

Experimental Animals

A total of 15 adult male donkeys with an average weight of 100.1 ± 1.29 kg, aged between 4 – 5 years and belonging to the Equine and Camel Research Programme, NAPRI, Shika- Zaria Nigeria were used for the study. Ten (10) of them were apparently healthy and devoid of helminth infection confirmed via faecal analysis in the helminthology laboratory, Department of Veterinary Parasitology, Faculty of Veterinary Medicine, ABU, Zaria Nigeria. The other five were infested with roundworm (Strongyles), Ivermectin® at a dose rate of 0.2 mg/kg per os was administered to the infested donkeys to ensure complete helminth-free condition in the donkeys before commencement of the research.

Experimental design and treatments

Fifteen of the donkeys were divided into three groups: A, B and C each containing five donkeys. Each donkey in all the three groups were engaged in packing kg of load covering a distance of 10 km. No treatment was given to Group A, while Groups B and C received 7.5 mg/kg of levamisole per os.

Measurement of body weight and packing

Body weights were measured using an Avery weighing scale (W & T Avery Ltd United states of America) for large animals in the animal pen at Dairy Research Programme, NAPRI, Shika-Zaria. Packing was performed for 10 km (5 km going and 5 km returning), between 6 and 11 am in the morning to allow laboratory analysis of blood samples collected within the same day.

Training of the donkeys on load carrying

The donkeys were trained for a period of four weeks on load carrying before commencement of the experiment. The estimated weight of load (sand), which was 40 % (40 kg) of each donkey's body weight was placed in "mangala" bags which are specially designed for load carrying by pack animals.

Determination of vital parameters

Respiratory rate (cycles/min), pulse rate (beats/min) and rectal body temperature (^{o}C) were determined by the method described by Mealey (2019). Respiratory rate was determined by gently placing a stethoscope on the thoraco–lumbar region to auscultate respiratory sounds and movements (for one minute), the pulse rate was counted by gently placing one or two fingers to feel the pulse on the mandibular artery for one minute, and a digital rectal thermometer (iProven manufactured in the United States of America) was used to measure the body temperature by gently placing it on the mucosa of the rectum of each donkey for one minute.

From each donkey, five millilitres of blood sample was collected via jugular venepuncture, and 2 mL was placed into potassium ethylenediamine tetra-acetic acid vacutainers. The remaining 3 mL was placed in test-tube and allowed to clot. The 2-mL blood sample was used for blood cellular analysis while the harvested serum was used for biochemical analyses. Faecal analysis was carried out using the McMaster technique with the 5 g faeces collected form each donkey into sterile polythene bags (Cringoli et al. 2010).

The vital parameters and blood collection were carried out before packing and upon return from packing between 6 and 11 am in the morning.

Determination of haematological parameters and erythrocyte osmotic fragility (EOF)

Blood cellular components of packed cell volume (PCV), red blood cell counts (RBCs), absolute and differential leucocyte counts were determined as described by Schlam et al. (1975). Erythrocytic indices were calculated using standard formulae.

Erythrocyte osmotic fragility was determined as described by Faulkner and King (1970). Briefly, 0.02 mL of whole blood was placed in each of 6 tubes, containing increasing concentrations; 0, 0.1, 0.3, 0.5, 0.7, 0.9% of phosphate-buffered sodium chloride (NaCl) solution at pH 7.4. Thereafter, the contents of the tubes were gently mixed and incubated at room temperature (37 ^{0}C) for 30 minutes and centrifuged at 1500 g per minute for 10 minutes. Optical density of the supernatant was determined spectrophotometrically at 540 nm using a spectrophotometer (Spectrolab 23 A®, Labomed Incorporated, Culver City, California, USA). Haemolysis in each tube was expressed and calculated as a percentage, assuming haemolysis in distilled water (0% NaCl) as 100%.

Biomarkers of oxidative stress

The concentration of malondialdehyde (MDA) and activities of Superoxide dismutase (SOD) and Catalase (CAT) were determined as described by Okhawa et al. (1979) for MDA, Fridovich (1989). for SOD and Aebi (1974) spectrophotometric method for Catalase.

Data Analyses

The data obtained were expressed as mean ± SEM. Values were subjected to two-way analysis of variance (ANOVA) followed by Tukey's post-hoc test to compare the differences between the means. Values of $P < 0.05$ were considered as significant.

Results

There was no significant ($P > 0.05$) difference between and within groups in the values of the respiratory rate, pulse rate and rectal body temperature but the post-packing values were higher ($P > 0.05$) compared to the pre-packing values (Table I)

Blood cellular components and biomarker of oxidative stress

Values of the blood cellular parameters determined did not differ significantly ($P > 0.05$) (Table II). The values of the oxidative stress parameters were also not significant ($P > 0.05$) although post-packing values of MDA were higher ($P < 0.05$) when compared to Pre-packing values. The post-packing activities of antioxidant enzymes SOD and catalase were not different ($P > 0.05$) from the pre-packing ones. (Table III)

Effect of levamisole in donkeys on erythrocyte osmotic fragility post-packing

The EOF was highest in donkeys infested with Strongyles and administered levamisole and subjected to packing. This group was closely followed by donkeys administered levamisole only and subjected to packing. The donkeys that were not treated with levamisole but subjected to packing had the least of EOF compared to any other group. (Figure. I).

Discussion

levamisole administered at 7.5 mg/kg body weight per os had no significant effect on the vital parameters (respiratory rate, pulse rate and rectal temperature), of the treated groups (pre- and post-packing). The relative increase in RR, PR and RT post-packing in all the groups may be due to stress induced by packing the donkeys for 10 km, but this packing distance was, apparently, not enough to induce any significant change in the parameters post-packing. The observed, mild increases across all the treated groups in the rectal temperature values may be due to the ability of levamisole to cause increase in body temperature (pyrexia). Gupta and Gupta (2003) reported that levamisole has the ability to cause pyrexia when given at therapeutic doses in man although the exact mechanism by which this is effected remains unknown. The level of exertion that the donkeys were exposed to due to packing could have caused the mild increases in all the vital parameters for the packed donkeys.

The total white blood cell count increased across the groups, though the differences were not significant. This finding could be due to levamisole's activity on cell-mediated immunity, mainly as a booster. It may also be due to the cholinergic activity of levamisole on the leucocytes and its ability to effect the maturation of immature leucocytes and stimulate T-cell differentiation (Wauwe and Janssen 1991; Mohammed et al. 2009). Furthermore, the increase in the red blood cell and leucocyte counts post-packing may be due to exercise-induced release of blood from splenic reserve into peripheral circulation (Bizjaka et al. 2020). The observed mild lymphocytosis in all the packed donkeys may be due to the effect of levamisole known to increase protein and nucleic acid synthesis in resting lymphocytes leading to lymphocytosis (Bourne et al. 1978 and Olusi et al.1979) reported similar effects of levamisole on the immune responses of experimentally malnourished rats while Mohammed et al. (2009) showed the beneficial effect of immunomodulation with levamisole on the course and pathogenesis of acute experimental *Trypanosoma congolense* infection in sheep.

The observed insignificant changes in the red blood cell count, packed cell volume, haemoglobin concentration and erythrocytic indices (MCV, MCH, MCHC) between treated and un-treated groups showed that immune-modulation with levamisole has no effect on the parameters as also reported by Mohammed et al. (2009) and Chethan et al. (2019) levamisole has been shown to boost the immune system in disease conditions, usually in more severe stressed states, as reported by Chethan et al. (2019), who demonstrated the booster effect of levamisole in diarrhoea due to Rotavirus infection in pigs.

There was increase in MDA concentration in both control group (A) and treatment groups (B and C) after packing. This may be due to free-radical induced lipid peroxidation of erythrocytic membranes; which has been shown to damage affected cell integrity thus leading to cell destruction (Jain 1986; Hanzawa and Wantanabe 2000). No significant change was observed in catalase, MDA concentration and Superoxide dismutase levels between treated and un treated groups. This result may be due to the protection and amelioration of stress by levamisole on erythrocyte membrane from lysing and reduction in the protection of the enzymatic activities of SOD and catalase. This finding agrees with the report of Aly et al. (2012) on the increase in the parameters due to levamisole treatment in rats subjected to only severe stress/disease conditions. Thus the 10 km packing with 40 kg load was not stressful enough in the donkeys too exert

significant changes in all of the parameters. There is need for further or higher loads evaluation to show the limit of load packing in the donkeys.

The results shows that the EOF was least in donkeys that weren't administered levamisole and subjected to packing. Thus, it appears that levamisole enhanced the fragility if red blood cells in the treated groups at 0.3% NaCl concentration. At other percentages of NaCl concentration the Erythrocyte osmotic fragility percentage did not differ among groups. The findings of the present study which shows that levamisole may increase fragility of the erythrocyte membrane and thus enhance their lyses under hypotonic conditions although Yaqub et al. (2014) and Olaifa et al. (2015) in donkeys exposed to packing and road transport have demonstrated an increase in erythrocyte osmotic fragility but Ascorbic acid was used in both cases as an antioxidant. The current study shows that levamisole did not ameliorate stress due to packing and helminth infestation. Apparently, levamisole may not be a potent antioxidant involved in scavenging oxidants generated in donkeys following their exposure to packing and helminth infestation. Therefore, donkeys who are to be transported especially those infested with *Strongylus* spp, may require the administration of additional agents such as ascorbic acid exhibiting antioxidant activity in order to ameliorate the oxidative stress induced damages to cell membrane. Furthermore, it appears that the distance of 10 km covered during the packing of the donkeys may not be adequate to induce stress response manifested in oxidative stress changes in the packed donkeys. This requires further investigation.

Conclusion

Packing of donkeys for 10 km did not induce significant changes in blood cellular components and biomarker of oxidative stress, but levamisole improved erythrocyte membrane stability.

Recommendations

Levamisole may be used to ameliorate stress at a dose rate of 7.5 mg/kg in packed donkeys carrying 40 kg load for 10 km. Further studies involving durations longer than 10 km and packing load weight above 40 kg are required to assess the actual effect of levamisole in alleviating stress in donkeys. Seasonal variations (rainy, cold-dry and hot-dry seasons) in the ameliorative effects of stress due to packing should be carried out to enable adequate assessment of levamisole ameliorative effect on stress during the hot-dry season.

Statements And Declarations

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All authors certify that they have no affiliations with or involvement in any organization or entity with any financial interest or non-financial interest in the subject matter or materials discussed in this manuscript.

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Data Availability

Not Applicable

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Tables

Due to technical limitations, Tables 1,2 and 3 are only available as a download in the Supplemental Files section.

Figures

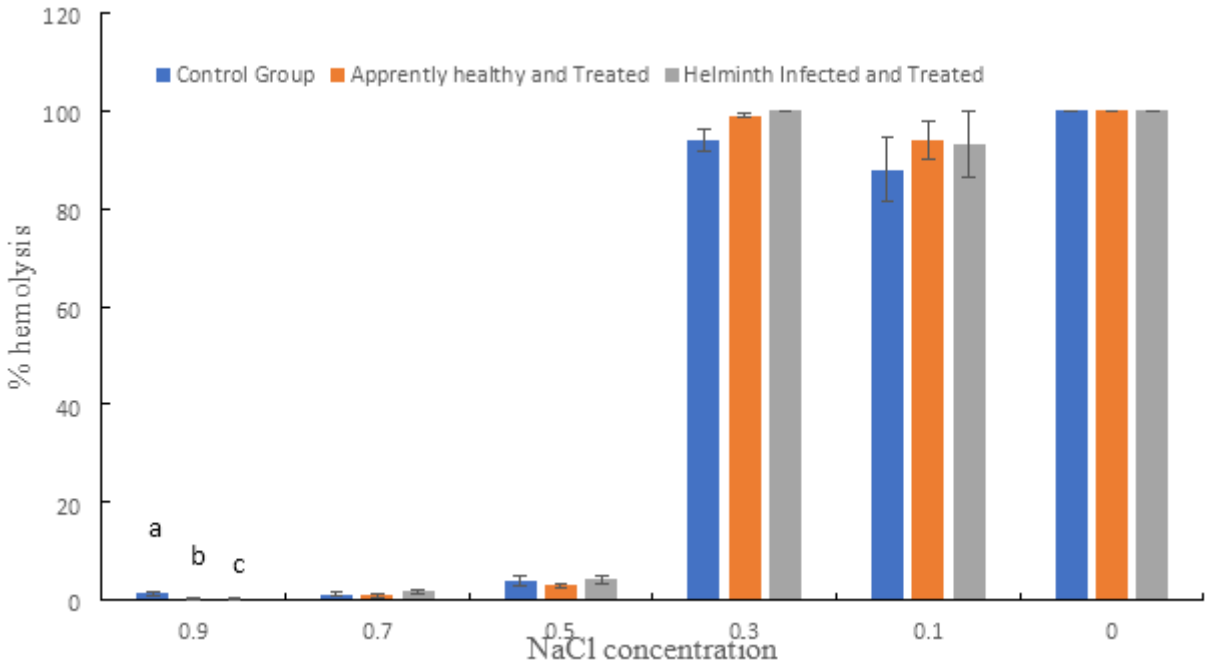

Figure 1

Effect of packing and levamisole erythrocyte osmotic fragility in donkeys, Error bars with superscripts abc are significantly ($P < 0.05$) different

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- TableI.docx
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- TableIII.docx