Journal of Veterinary and Biomedical Sciences Vol. 6 (2) 60 – 76, 2024
ISSN: 2659 – 0743
DOI: 10.36108/jvbs/4202.60.0270

AMELIORATIVE EFFECTS OF DIMETHYL SULPHOXIDEAND FISETIN ON OXIDATIVE STRESS BIOMAKERS IN LAME HORSES

Sambo, K.W [1], Saidu, S.N.A [2] , Sackey, A.K.B [2] , Suleiman, M.M [3] , Andrew, A [4], Karaye, G.P.[5] Idoko, S.I [6], Edeh, E.R[5] and Ijoh, B.B[6]

[1]Veterinary Teaching Hospital, Equine Unit, Ahmadu Bello University, Zaria.

[2]Department of Medicine, Faculty of Veterinary Medicine, Ahmadu Bello University, Zaria.

[3]Department of Pharmacology, Faculty of Veterinary Medicine, Ahmadu Bello University, Zaria.

[3]Department of Veterinary Surgery and Radiology, Faculty of Veterinary Medicine, Ahmadu Bello University, Zaria.

[5]Department of Veterinary Medicine, Surgery and Radiology, Faculty of Veterinary Medicine, University of Jos, 930222, Nigeria.

[6]Department of Veterinary Pathology, Faculty of Veterinary Medicine, University of Abuja, Gwagwalada, 902101, Nigeria..

[6]Department of Veterinary Parasitology and Entomology, University of Maiduguri, Nigeria.

s

Correspondence: email:2wonderksambo2@gmai.com; Tel: +234-8054-5544-81.

ABSTRACT

Lame horses are more susceptible to oxidative stress, an imbalance in the oxidant/-antioxidant system in cells. The aim of this study is to evaluate the effects of Dimethyl Sulphoxide and fisetin in combating oxidative stress, and biomarkers in lame horses. Dimethyl Sulphoxide and fisetin were administered singly, in half doses and in combination to lame horses, their activities on antioxidant enzymes (catalase, glutathione peroxidase, and superoxide dismutase) and malondialdehyde were observed. Twenty-five lame horses, numbered and selected using purposive simple random sampling without replacement, were distributed into five groups (A-D) of five horses each showing clinical signs of lameness (Graded from 0 to 2). Dimethyl Sulphoxide (1 mg/kg) was administered through intravenous injection for each horse once per week while fisetin was administered per os at 10 mg/kg and given daily for 60 days. The result showed that there was a significant reduction in MDA concentration from 737.22 ± 0.63 nmol/mg to 216.0.76 nmols/mg, and SOD activity was increased from 11.12± 0.07 to 12.10 ± 0.20, While CAT activity was increased significantly from 39.88± 0.48 IU/L to 62.44 ± 0.58 IU/L. GPx was reduced from 17.62 ± 0.38 IU/L to 17.46 ± 1.44 IU/L. There was a significant decline in the oxidative stress biomarker in lame horses with administration of dimethyl sulphoxide and fisetin.

Key words: lame horses, oxidative stress, Dimethyl Sulphoxide, fisetin.

Introduction

Lameness is one of the most common conditions that affect horses [1] it is a term used to describe the horse's gait being adversely affected by pain or a restriction in range of movement [1]. Causes of lameness include upward fixation of the patella, femoral chip fracture, olecranon bursitis, bone spavin and puncture wounds of the hoof to name the least. There are a range of diagnostic tools, such as radiography, ultrasonography, nuclear scintigraphy, computed tomography (CT), magnetic resonance imaging (MRI), thermography, arthroscopy and lameness locator [2] that are used for clinical observation during diagnoses of lameness. Most of the drugs used in the treatment of lameness are limited in efficacy and associated with the gastro-intestinal side effects which are mainly ulcers and colic [3]. Hence, the use of Dimethyl sulphoxide which is an organic sulphur compound and fisetin, a flavonoid group of polyphenols may be of great benefit in mitigating both the adverse effects of oxidative stress and in the intervention of lameness in horses.

A lameness grading system has been developed by the American Association of Equine Practitioners (AAEP) [4]. The scale ranges from zero to five, with zero being no perceptible lameness, and five being most extreme.

Oxidative stress is a detrimental imbalance in the oxidant-antioxidant system of cells which is exacerbated in lame horses [5]. All cells in the body have antioxidant defence systems, which are separated into the enzymatic and non-enzymatic systems capable of alleviating the deleterious effects of these ROS. The enzymatic group includes the superoxide dismutase (SOD), catalase (CAT) and glutathione peroxidase (GPx) enzymes. Catalase plays an important role in the elimination of hydrogen peroxide and promotes its catalysis to water. Glutathione peroxidase GPx converts reduced glutathione (GSH) to oxidised glutathione (GSSG), by removing hydrogen peroxide and forming water. Superoxide dismutase catalyses the dismutation of superoxide into oxygen and hydrogen peroxide. But when the production of free radicals exceeds the cellular antioxidant capacity, oxidative stress occurs and can cause muscle disorder and fatigue, as well as a number of diseases[6] This oxidative stress, may cause DNA, protein, and lipid damage, resulting to changes in chromosome instability, genetic mutation, and modulation of cell growth that may lead to cancer [7]. Dimethyl Sulphoxide Dimethyl Sulphoxide was first synthesised in the nineteenth century by the Russian chemist Alexander Saytzeff [8] due to its pharmacological and therapeutic properties, Dimethyl sulphoxidehas been used in the field of medicine.

Dimethyl Sulphoxidealso has anti-inflammatory properties [9] including a myorelaxant effect [8] and can also preserve the stability of lysosomal membranes [10]. It is noteworthy that Dimethyl sulphoxide is able to cross any barrier, such as the placental barrier [10], blood-brain barrier [10], or the intact bladder wall [10]. It is among the most potent free radical scavengers known to man [11].

Another type of free radical scavengers are the flavonoids, example of which is fisetin, which

belong to a group of natural substances which are found in fruit, vegetables, grains, bark, roots, stems, flowers, tea, and wine [12]. An important property of fisetin is the scavenging of free radicals. In vitro experimental also showed that fisetin possess anti-inflammatory, antiallergic, antiviral, and anticarcinogenic properties [13]. The best-described property of almost every group of flavonoids is their capacity to act as antioxidants. This cellular damage causes a shift in the net charge of the cell, changing the osmotic pressure, leading to swelling and eventually cell death. Free radicals can attract various inflammatory mediators, contributing to a general inflammatory response and tissue damage. To protect themselves from reactive oxygen

species, living organisms have developed the anti-oxidant defense mechanisms [14].

Materials and Methods

The study was carried out in a private polo farm in Igabi Local Government Area, located at 10º 29' N, 07º 28' E, of Kaduna State, Nigeria. The ethical clearance for this work was obtained from the Committee for Animal Use and Care (ABUCAUC) with Ref No: ABUCAU/2021/091.

Experimental Drugs and Kits

Dimethyl Sulphoxide was purchased from Ekinos® Products Veterinarios Para Equino Deportivo, Rio De Janerio, Brazil. Fisetin was also purchased from CN-Lab, Flavonoid supplement Aliexpress ™, China; Malonaldehyde, superoxide dismutase and glutathione kits was purchased from Northwest Life Science Specialist, Vancouver, Canada; while the Catalase kit was purchased from Abcam PLC,330 Cambridge Science Park, Cambridge, United Kingdom.

Experimental Animals

The simple random sampling without replacement was used to select the twenty five horses showing clinical signs of lameness [2]. The horses were housed in standard horse stables (measuring 10m x 2m) made of concrete floor with saw mills as bedding materials, cement block wall and zinc roof and well ventilated.

The horses were pre-conditioned for two weeks before the commencement of the study, during which they were screened and treated for external, intestinal and haemo-parasites using multiworm plus ^{M} oral paste (Abamhjyectin 3.7 mg/kg and Praziquantel 4.6 mg/kg, Equifox Veterinary Products, South Africa), and imidocarb (4 mg/kg, intramuscularly) respectively. Similarly, cypermectin-pour-on 250 mg/kg (topically), was used to treat external parasites on the horses.

Treatment Groups

Dimethyl Sulphoxide (1mg/kg) was administered through intravenous injection for each horse once per week (based on manufactures' instruction) in order to evaluate the ameliorative effect of the drugs on oxidative stress in the lame horses. Fisetin was administered *per os* at 10 mg/kg and given daily for sixty days, to achieve the same objective as dimethyl su;phoxide mentioned above.

The lame horses in the stables were numbered and selected using simple random sampling without replacement into five groups of five horses each (A, B, C, D and E):

1. Group A served as untreated control but administered with distilled water,
2. Group B horses were administered with full standard dosage of Dimethyl Sulphoxide (1 mg/kg),
3. Group C horses were given full standard dosage of fisetin at 10 mg/kg,
4. Group D horses were placed on half-standard dosage of both Dimethyl Sulphoxide (0.5 mg/kg) and fisetin (5 mg/kg),
5. while group E horses were administered with standard doses of both Dimethyl Sulphoxide(1 mg/kg) and fisetin (10 mg/kg).

The daily rectal temperatures, pulse and respiratory rates were taken, as described by Nettie *et al*., [15]. 10 ml of blood was allowed to stand for 30 minutes to clot and thereafter centrifuged at 1000 *g* for 30 minutes. The resultant serum was decanted and stored at -20˚C until used later for the determination of the concentrations of MDA, SOD, (CAT) and GpX.

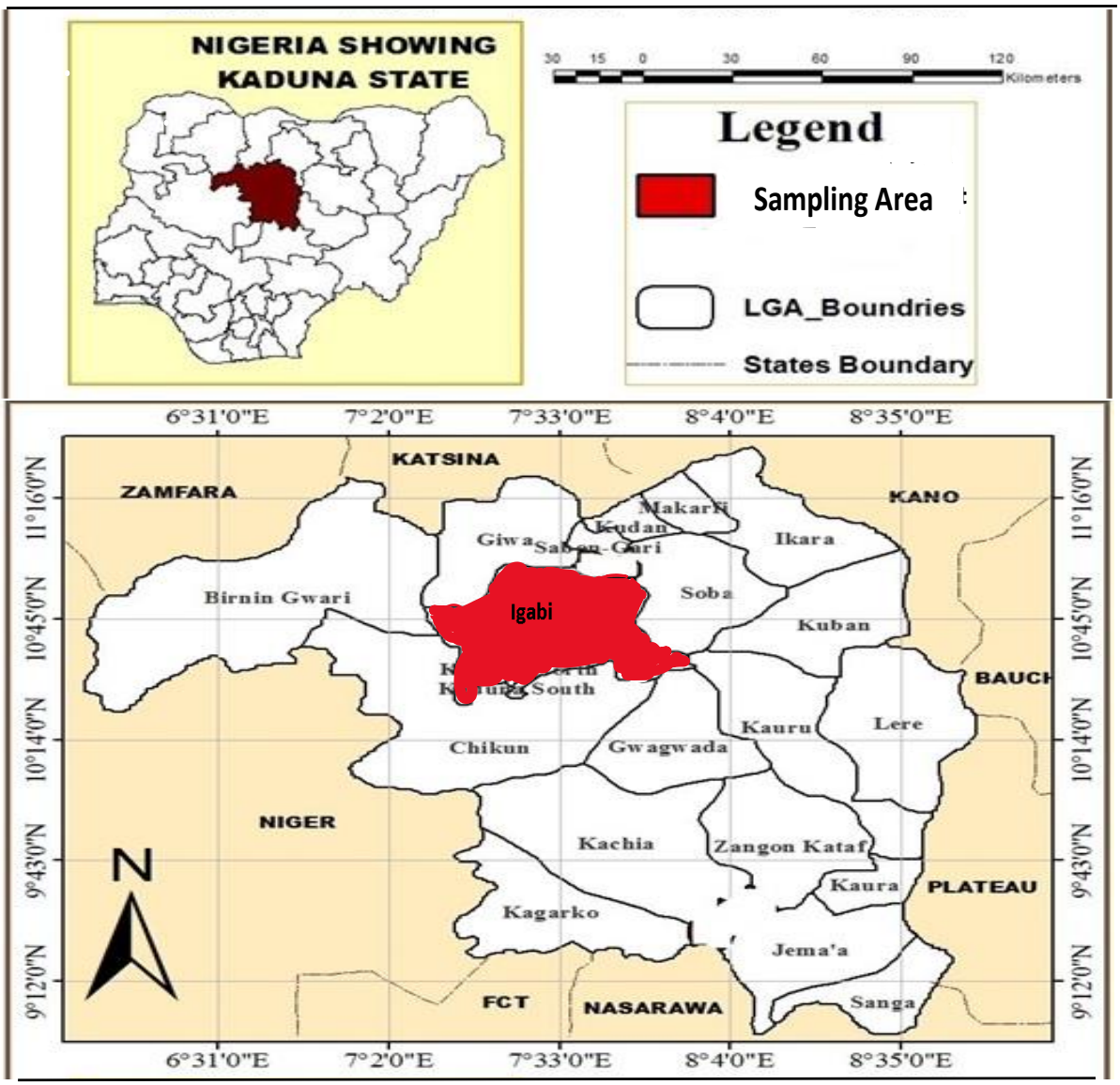

Figure 1a: Map of Nigeria showing Kaduna State

b: Map of Kaduna State showing Igabi Local Government Area

Laboratory Investigations

Determination of MDA Concentrations was done as described by Botsoglou [16], SOD was done as described by Martin *et al.* [17], CAT was done as described by Aebi, [18]. While GPx was done as described by Flohe and Gunzler, [19].

Data Analysis

Data obtained were expressed as mean ± standard error of mean (mean ± sem). The relationship between the effects of Dimethyl sulphoxide and fisetin in each

group was assessed using repeated two-way analysis of variance (ANOVA), followed by Tukey *post hoc* test. Values of $p < 0.05$ were considered statistically significant. Statistical software used was graph pad prism version. 5.0.

Results

Effects of Dimethyl Sulphoxide Dimethyl Sulphoxide and Fisetin on Malondialdehyde (MDA)

Concentration in Lame Horses

The overall mean MDA concentration for horses in control (Group A was 836.40 ± 68.34 nmol/mg) was significantly ($p < 0.05$) higher compared to those in Groups B, C, D and E 670.30 ± 34.10 nmol/mg, 798.30 ± 14.00 nmol/mg, 631.70 ± 77.67 nmol/mg and 584.10 ± 59.45 nmol/mg respectively). Similarly, Group B had a significantly ($p < 0.05$) lower mean MDA concentration compared to animals in Group C. Also, Group C had a significantly ($p < 0.05$) higher mean MD concentration compared to those in Groups D and E, while those in Group D had a significantly $p < 0.05$) higher mean MDA concentration compared to Group E. (Table 1: Figure 2).

Effects of Dimethyl SulphoxideDimethyl Sulphoxide and Fisetin on Superoxide dismutate (SOD) activity in Lame Horses

The overall mean SOD activity for Group A (6.85 ± 1.05 IU/L) was significantly ($p < 0.05$) lower compared to Group B, C, D and E (9.02 ±1.03 IU/L, 8.37 ±0.62 IU/L, 9.00 ±0.33 IU/L and 10.13 ±0.58 IU/L respectively. (Table 2: Figure 3).

Effects of Dimethyl SulphoxideDimethyl Sulphoxide and Fisetin on Catalase (CAT) activity in Lame Horses

The overall mean CAT activity for Group A horses was higher (65.92 ±8.47 IU/L) when compared to Groups B, C, D and E (47.97 ± 6.65 IU/L, 62.54 ± 3.98 IU/L, 51.21 ± 6.19 IU/L and 54.72 ± 5.72 IU/L) respectively. (Table 3: Figure 4).

Effects of Dimethyl SulphoxideDimethyl Sulphoxide and Fisetin on Glutathione Peroxidase

(GPx) activity in Lame Horses

The overall mean GPx activity for Group A horses was highest (29.42 ± 5.91 IU/L) compared to those for Group B, C, D and E (19.04 ± 0.93 IU/L, 19.14 ± 1.19 IU/L, 18.78 ± 0.99 IU/L and 18.71 ± 1.19 IU/L). Groups D and E showed no statistically significant findings. (Table 4: Figure 5).

Table I: Mean ± SEM values of MDA (nmol/mg) for Lame horses, treated with Dimethyl sulphoxide and Fisetin for eight weeks, Groups A-E.

Groups	Week 1	Week 2	Week 3	Week 4	Week 5	Week 6	Week 7	Week 8	M
A	799.22 ± 3.42[a]	741.16 ± 0.87[a]	922.40 ± 0.29[a]	1244.76 ± 1.83[a]	901.86 ± 40.42[a]	583.04± 2.11[a]	832.82 ± 0.94[a]	723.86 ± 0.86[a]	836.40
B	737.22 ± 0.63[b]	855.02 ± 1.51[b]	1331.70 ±1. 03[b]	793.38 ± 1.13[b]	834.84 ± 0.51[b]	249.26 ± 4.29[b]	344.02 ± 0.93[b]	216.76 ± 0.60[b]	670.30
C	564.42 ± 1.13[c]	639.66 ± 1.28[c]	635.12 ± 0.62[c]	1003.74 ± 0.67[c]	894.12 ± 1.47[c]	493.64 ± 0.53[c]	677.76 ± 1.08[c]	1478.18 ± .89[c]	798.30
D	388.94 ± 2.81[d]	376.74 ± 2.27[d]	874.58 ± 1.70[d]	870.80 ± 2.29[d]	882.78 ± 1.78[d]	466.50 ± 2.05[d]	646.38 ± 1.62[d]	547.04 ± 2.46[d]	631.70
E	555.60 ± 3.02[e]	864.34 ± 0.79[e]	654.40 ± 1.22[e]	776.96 ± 1.27[e]	533.12 ± 0.99[e]	393.18 ± 1.58[e]	437.74 ± 1.24[e]	457.62 ± 0.95[e]	584.10

Table 2: Mean ± SEM values of SOD (IU/L) activity for Lame horses treated with Dimethyl sulphoxide and Fisetin for eight weeks, Groups A-E.

Groups	Week 1	Week 2	Week 3	Week 4	Week 5	Week 6	Week 7	Week 8	Mean
A	3.32 ± 0.20[a]	8.42 ± 0.31[a]	7.92 ± 0.13	8.80 ± 0.20[a]	1.20 ± 0.12[a]	6.96 ± 0.34[a]	8.88 ± 0.36[a]	9.28 ± 0.34[a]	6.85 ± 1.05
B	10.56 ± 0.18[b]	12.74 ± 0.47[b]	7.92 ± 0.19	12.58 ± 0.15[b]	3.86 ± 0.22[b]	8.16 ± 021[b]	7.52 ± 0.18[b]	8.80 ± 0.34[b]	9.02 ±1.03
C	5.86 ± 0.12[c]	7.64 ± 0.16	7.78 ± 0.11	8.56 ± 0.30	8.68 ± 0.14[c]	10.88 ± 0.21[c]	10.70 ± 0.15[c]	6.88 ± 0.18[c]	8.37 ±0.62
D	9.56 ± 0.10[d]	9.74 ± 0.19	8.46 ± 0.48	9.60 ± 0.18[d]	7.02 ± 0.17[d]	9.58 ± 0.21[d]	8.56 ± 0.30	9.46 ± 0.20	9.00 ±0.33
E	11.12 ± 0.07	8.68 ± 0.33	8.38 ± 0.31	10.70 ± 0.15[e]	7.82 ± 0.10	10.30 ± 0.19	11.94 ± 0.37[e]	12.10 ± 0.20[e]	10.13 ±0.58

Table 3: Mean ± SEM values of CAT (IU/L) activity for Lame horses treated with Dimethyl sulphoxide and Fisetin for eight weeks, Groups A-E.

Groups	Week 1	Week 2	Week 3	Week 4	Week 5	Week 6	Week 7	Week 8	Mean
A	78.90 ± 0.53[a]	71.78 ± 2.20[a]	71.82 ± 2.19[a]	84.52 ± 0.49[a]	59.08 ± 0.52[a]	93.72 ± 0.28[a]	17.24 ± 0.39[a]	50.28 ± 0.57[a]	65.92 ±8.47
B	58.08 ± 0.32[b]	74.30 ± 0.36	53.38 ± 0.60[b]	62.44 ± 0.58[b]	55.38 ± 0.69[b]	28.70 ± 1.19[b]	21.78 ± 0.54[b]	29.72 ±0.80[b]	47.97 ± 6.65
C	54.54 ± 0.52[c]	45.38 ± 0.58[c]	65.36 ± 0.52[c]	74.16 ± 0.21[c]	74.68 ± 0.81[c]	58.42 ± 0.58[c]	74.32 ± 0.72[c]	53.48 ± 1.03	62.54 ±3.98
D	55.38 ± 0.69	58.06 ± 0.43[d]	74.32 ± 0.72	53.58 ± 0.96[d]	62.74 ± 0.53[d]	55.38 ± 0.69	28.96 ± 0.70[d]	21.26 ± 0.85[d]	51.21 ±6.19
E	39.88 ± 0.48[e]	28.82 ± 1.21[e]	45.66 ± 0.60[e]	74.68 ± 0.81	58.42 ± 0.58	74.32 ± 0.72[e]	53.52 ±0.97[e]	62.44 ± 0.58[e]	54.72 ±5.72

Table 4: Mean ± SEM values of GPx (IU/L) activity for Lame horses treated with Dimethyl sulphoxide and Fisetin for eight weeks, Groups A-E.

Groups	Week 1	Week 2	Week 3	Week 4	Week 5	Week 6	Week 7	Week 8	Mean
A	15.42 ± 0.55[a]	17.36 ± 0.43[a]	20.44 ± 0.71[a]	31.70 ± 0.29a	24.66 ± 0.68[a]	28.12 ± 1.38[a]	68.18 ± 0.27[a]	29.44 ± 0.39[a]	29.42 ± 5.91
B	17.62 ±0.38[b]	17.96 ± 0.34	18.30 ± 0.38[b]	18.08 ± 0.34[b]	17.48 ± 0.39[b]	25.20 ± 0.64[b]	20.22 ± 0.30[b]	17.46 ± 0.44[b]	19.04 ± 0.93
C	17.10 ± 0.46	13.22 ± 0.41[c]	18.22 ± 0.42	20.04 ±0.36	19.70 ± 0.77[c]	19.42 ± 0.31[c]	25.20 ± 0.76[c]	20.22 ± 0.46[c]	19.14 ± 1.19
D	18.08 ± 0.34	17.36 ± 0.37	17.56 ± 0.43	25.20 ± 0.64[d]	20.22 ± 0.41	17.46 ±0.44	16.84 ± 0.40[d]	17.48 ± 0.30	18.78 ± 0.99
E	19.20 ± 0.86	13.36 ± 0.44	19.24 ± 0.89	18.48 ±0.38	16.54 ± 0.34	25.20 ± 0.64	20.22 ± 0.46	17.46 ± 0.44	18.71 ± 1.19

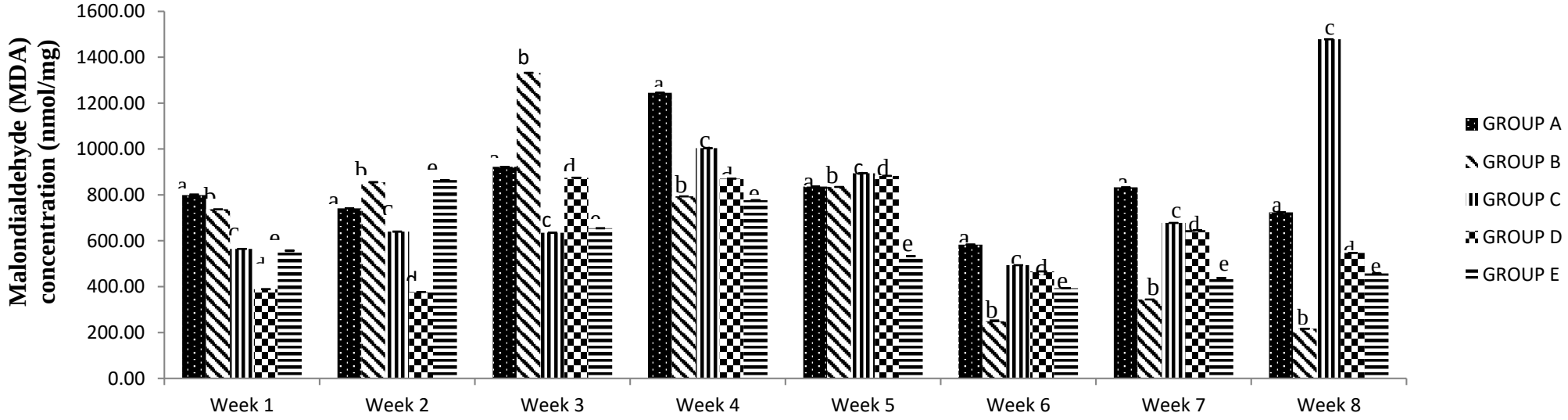

Figure 2: Effects of administration of Dimethyl sulphoxide and Fisetin on Malondialdehyde concentration in Lame horses following eight weeks of treatment

$^{a, b, c, d, e}$ = The different letters designated within each week indicates significant difference within the groups means ($p < 0.05$).

Key: Group A- Control (2 ml distilled water) *per os*

Group B- Dimethyl Sulphoxide (1 mg/kg) i/v

Group C- Fisetin (10 mg/kg) *per os*

Group D – Dimethyl sulphoxide 0.5 mg/kg) i/v and Fisetin (5 mg/kg) per os

Group E- Dimethyl sulphoxide (1 mg/kg) i/v and Fisetin (10 mg/kg) per os

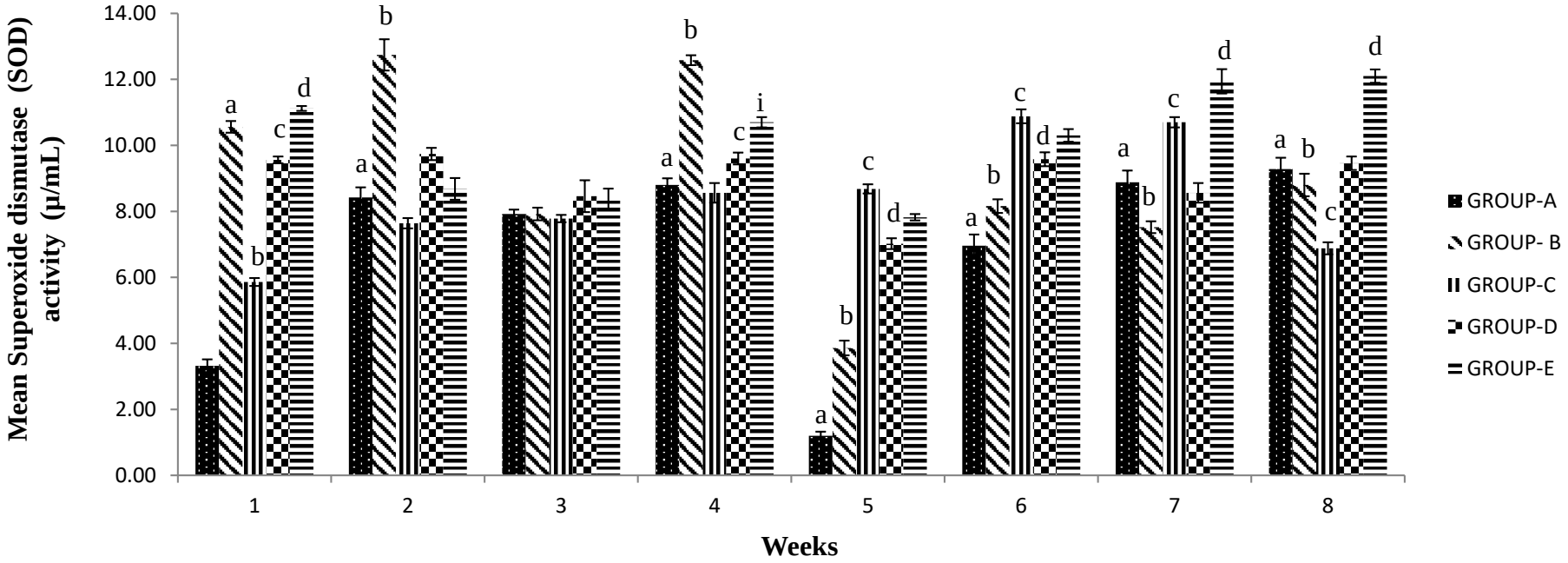

Figure 3: Effects of SOD activity in Lame horses following treatment with Dimethyl sulphoxide and Fisetin for eight weeks.

$^{a, b, c, d, e}$ = The different letters designated within each week indicates significant difference within the groups means ($p < 0.05$).

Key: Group A- Control (2 ml distilled water) *per os*

Group B- Dimethyl Sulphoxide (1 mg/kg) i/v

Group C- Fisetin (10 mg/kg) *per os*

Group D – Dimethyl sulphoxide 0.5 mg/kg) i/v and Fisetin (5 mg/kg) per os

Group E- Dimethyl sulphoxide (1 mg/kg) i/v and Fisetin (10 mg/kg) per os

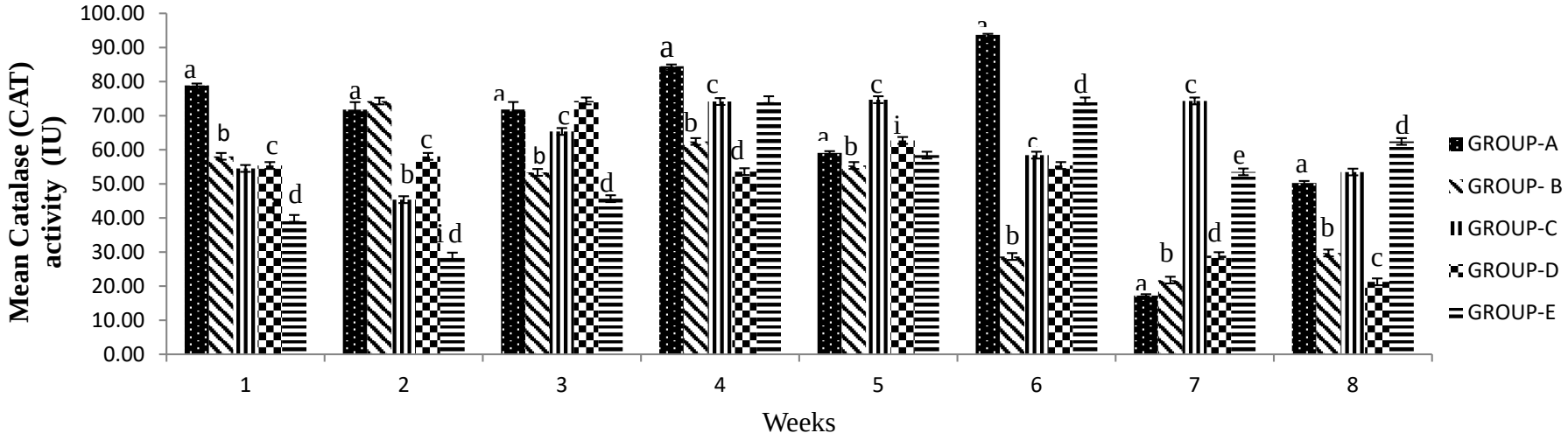

Figure 4: Effects of CAT activity in Lame horses following treatment with Dimethyl sulphoxide and Fisetin for eight weeks.

[a, b, c, d, e] = The different letters designated within each week indicates significant difference within the groups means ($p < 0.05$).

Key: Group A- Control (2 ml distilled water) *per os*

Group B- Dimethyl Sulphoxide (1 mg/kg) i/v

Group C- Fisetin (10 mg/kg) *per os*

Group D – Dimethyl sulphoxide 0.5 mg/kg) i/v and Fisetin (5 mg/kg) per os

Group E- Dimethyl sulphoxide (1 mg/kg) i/v and Fisetin (10 mg/kg) per os

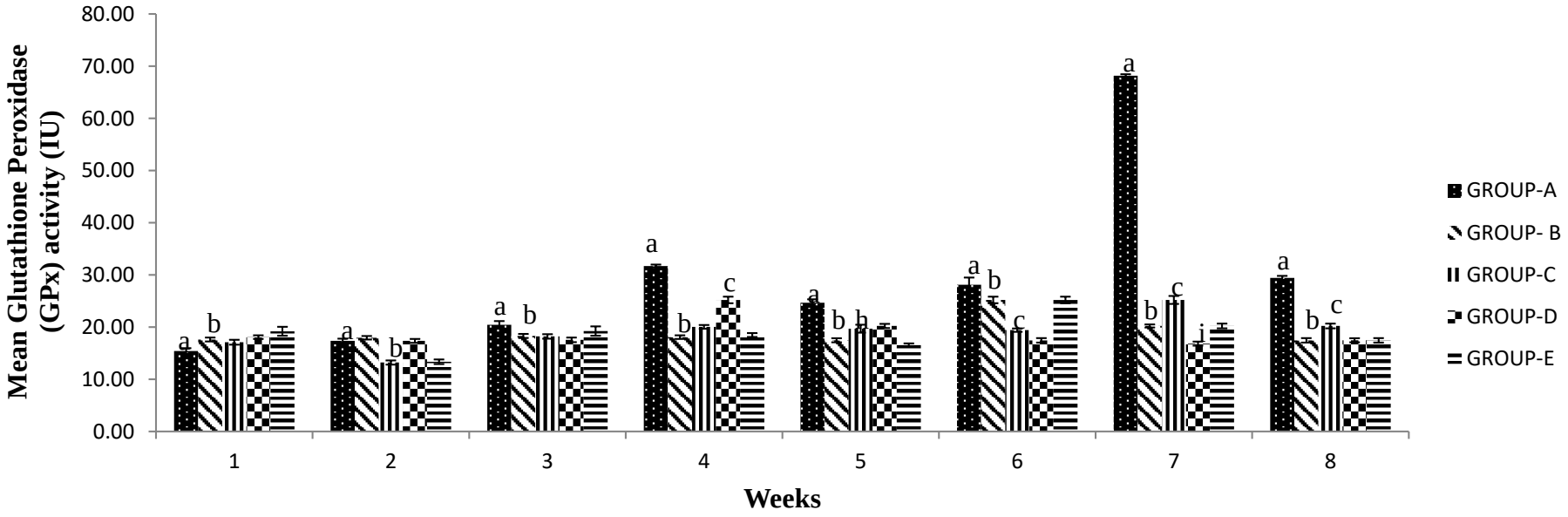

Figure 5: Effects of GPx activity in Lame horses following treatment with Dimethyl sulphoxide and Fisetin for eight weeks.

$^{a, b, c, d, e}$ = The different letters designated within each week indicates significant difference within the groups means ($p < 0.05$).

Key: Group A- Control (2 ml distilled water) *per os*

Group B- Dimethyl Sulphoxide (1 mg/kg) i/v

Group C- Fisetin (10 mg/kg) *per os*

Group D – Dimethyl sulphoxide 0.5 mg/kg) i/v and Fisetin (5 mg/kg) per os

Group E- Dimethyl sulphoxide (1 mg/kg) i/v and Fisetin (10 mg/kg) per os

Discussion

There was a significant decline in the oxidative stress biomarker (malondialdehyde concentration) which was ameliorated in the Group B, accompanied by Groups E and D horses respectively. The most prominent decrease after eight weeks of treatment was observed in Group B, which could be attributed to the anti-oxidant effects of dimethyl sulphodide [20]. This was then followed by the Group E combined at full standard doses, and probably due to the anti-oxidant properties they possess [20, 21]. However given singly, dimethyl sulphoxide had a higher decrease than fisetin, which could indicate that dimethyl sulphoxide has a more potent anti-oxidant effect when used singly than combined with fisetin. The decrease in MDA concentration in Group C at six weeks of treatment and subsequent increase at eight weeks of treatment, agrees with findings of Klaudia *et al*. [22]. Who reported that slight prooxidant properties of fisetin activates antioxidant systems including antioxidant enzymes. This observation agrees with findings of Prochazkova *et al*. [23] further confirmed by the reports of Fatin *et al.* [24] who reported fisetin as a potent antioxidant agent.

In this study, increase in SOD activity post treatment in Groups E and D horses (with dimethyl sulphoxide and fisetin) demonstrates the anti-oxidant effects of dimethyl sulphoxide and fisetin in ameliorating oxidative stress as a result of production of MDA [20, 21]. This result suggests that a combination of DMA and fisetin at full standard dose (dimethyl sulphoxide 1mg/kg and fisetin 10 mg/kg) is better at increasing the concentration of SOD activity and thus recommended, other than the combined administration at half standard doses. This in SOD activity may be because it is one of the front line defense anti-oxidant enzyme system against ROS in high oxidant environment potentiated by lameness [25].

Similarly, there was an observed increase in CAT activity in Group E after eight weeks of treatment, which is at variance with reports by Paulo *et al*., [26]. This increase could possibly be due to the drug delivery, penetration and potentiation power of dimethyl sulphoxide when used in combination as was done in this study [9]. The result of this study also showed that treatment with combined full standard dose of dimethyl sulphoxide fisetin caused higher CAT activity in the lame horses as exhibited by Group E, which was then followed by Groups D horses. The decrease in GPx activity after eight weeks of treatment could be attributed to the lack of exercise by all the lame horses, which has been reported to cause a decline in GPx level [27]. Furthermore, exposure to constant stress as the case is with these lame horses, may be responsible for decline in GPx activity, as was reported by Moffarts *et al*., [28, 29].

The result of this study has shown that fisetin administered alone in Group C horses, tends to enhance Gpx activity more in lame horses. This was then followed by the administration dimethyl sulphoxide alone as observed in Group B horses.

Conclusion

Administered singly, dimethyl sulphoxide appears to be more effective in decreasing MDA concencentration in the lame horses, following eight weeks of treatment, while administration of full standard dose singly, of dimethyl sulphoxide and fisetin reduced SOD activity. However, administration of combined half standard dose of dimethyl sulphoxide and fisetin reduced CAT activity and the administration of full standard dose singly of dimethyl sulphoxide (1mg/kg) and fisetin (10 mg/kg) reduced GPx activity.

REFERENCES

1. Kufa, M. (2019). Review on Lameness in Equine, *International Journal of Advanced Research in Biological Sciences*, 6(10): 101-109.

2. Aagje, M. H., Agneta, E., Filipe, M. S. B., Marc, H. W. K., Jan-Hein, S., Lars, R., Rene, W. and Anna, B. (2022). Movement asymmetries in horses presented for prepurchase or lameness examination, *Equine Veterinary Journal*, 54(2):334-346.

3. Vishwakarma, R.K. and Negi, D.S. (2020). The development of cox-1 and cox-2 inhibitors: a review. *International journal of pharmaceutical sciences and research*, 2: 3544-3555.

4. Helene, T. and Lucinda, M. (2021). Can sedation be used for equine lameness investigation? *Veterinary Evidence,* 6 (2): 2-13.

5. Saori, S., Azusa, G., Fumiko, M., Kimihiro, O. and Mariko, M. (2020). The Oxidative StressMarkers of Horses—the Comparison with Other Animals and the Influence of Exercise and Disease. *Animals* 10 (617): 2- 9.

6. Steven, J. F., Daniel, S. K., Marina, S. H., Qian, X. and Kathy, K. G. (2018). Reactive Oxygen Species in Metabolic and Inflammatory Signaling. *Circulation Research*. 122 6):877–902.

7. John, H. and Albena, D. (Oxidative Stress in Cancer, *Cellular Medicine*, 38 (2): 167-197.

8. Madruga, G. M., Crivellenti,L. Z., Borin-Crivellenti, S., Cintra, C, A, Gomes, L. G. and Spiller, P, R. (2017). Comparative use of Dimethyl SulphoxideDimethyl Sulphoxide in diff erent animal species. *Veterinarni Medicina*, 62, (04): 179–185.

9. Alexander, S., Goydyk, V.S., Gusakovsky, S.S. and Viacheslav, M.G. (2021). Modern aspects of the use of dimethyl sulfoxide Dimethyl Sulphoxide. P*harmacologyonline* 1:82-89.

10. Beatrice, G., Zehra, K., Beth, M., Bob, D. L., Stefano, P., Jeremy, M., Assunta, M., Francesca, P., Paola, S. and Peter, G. P. (2020). Effect of DIMETHYL SULPHOXIDEon the Mechanical and Structural Properties of Model and Biological Membranes. Biophysical Journal, Volume 119, Issue 2, 21 July 2020, Pages 274-286.

11. Morita, M., Naito, Y., Yoshikawa, T. and Niki, E. (2017). Antioxidant capacity of blueberry extracts: Peroxyl radical scavenging and inhibition of plasma lipid oxidation induced by multiple oxidants. *Journal of Berry Research*, 7(1): 1-9.

12. Patricia, H., Ludy, P. B. and Harold, R. (2019). Flavonoids: Potential Therapeutic Agents by Their Antioxidant Capacity. Health Benefits and Potential Applications. *Science Direct*, P: 265-288.

13. Prithviraj, K. (2019). Biological activities of flavonoids: an overview. *International Journal of Pharmaceutical Sciences and Research,* 10((4)):1567-1574.

14. Eva, T. and Filip, B. (2020). Oxidative Stress and Dietary Antioxidants: Free radicals: what they are and what they do. *Pathology*, P:3-13.

15. Nettie, L.,Karyn. M. and Carey,W.(2021). New Jersey Agricultural Experiment Station, Rutgers, Measuring Temperature, Pulse and Respiration (TPR): What's Normal for My Horse,. *Rutgen, cooperative extension fact sheet*, FS1262.

16. Bostglou, N.A. (1994). Rapid, sensitive and specific thiobarbituric acid method for measuring lipid peroxidation concentration in animal tissue, food and feedstuff samples. Journal of Agricultural and Food Chemistry, 42: 1931-1937.

17. Martin, J. P., Dailey, M. and Sagarman, E. (1987). Negetive and positive assay of superoxide dimutase based on haematoxylin autoxidation. Archives of Biochemistry and Biophysics. 255: 329-336.

18. Aebi, H. (1984). Catalase in vitro. *Methods of Enzymology*, 105: 121-126.

19. Flohe, L. and Gunzler, W.A. (1984). Assays of glutathione peroxidase. *Methods in Enzymology.* 105:114-121.

20. Eric, D. P. S., Cynthia, P. V., Joice, F., Sarah, R., Torquato, S., Fernando, M. J., Fernanda, R. A., Luís, C. L. C. and Raquel, Y. A. B. (2020). Effects of Joint Lavage with Dimethylsulfoxide on LPS-Induced Synovitis in Horses—Clinical and Laboratorial Aspects. *Veterinary Sciences,* 2:57.

21. Deepshikha, G. and Eksha, G. (2022). Flavonoids: Molecular Mechanism Behind Natural Chemoprotective Behavior-A Mini Review. *Biointerface Research in Applied Chemistry*, 12 (5): 5983 - 5995.

22. Klaudia, J., Lenka, H., Peter, L., Miriama, S., Saleh, H., Ibrahim, M., and Marian, V. (2019). Compounds Myricetin, Morin, 3′,4′-Dihydroxyflavone, Taxifolin and 4-Hydroxy-Coumarin in the Presence of Copper (II) Ions: A Spectroscopic, Absorption Titration and DNA Damage Study. Molecules 2019, 24(23), 4335; https://doi.org/10.3390/molecules24234335.

23. Procházkováa, D., Boušováb, I. and Wilhelmová, N. (2013).Antioxidant and prooxidant properties of flavonoids. *Fitoterapia,* 82 (4) : 513-523.

24. Fatin, F. J., Satirah, Z., Izatus, S. T., Zariyantey, A. H. and Siti, B. B. (2021). The Potential Role of Flavonoids in Ameliorating Diabetic Cardiomyopathy via Alleviation of Cardiac Oxidative Stress, Inflammation and Apoptosis. *International Journal of Molecular Sciences*, 22(5094) :1-22.

25. Younus, H. (2018). Therapeutic potentials of superoxide dismutase, *International Journal of Health Sciences,* 12(3): 88–93.

26. Paulo, C.L.S.,Eduardo, G.V., Débora, S., Luciano, A.S., Emilio, L.S., Marcos, M.P. and Ricardo, A.P. (2010). Effects of Therapeutic Pulsed Ultrasound and Dimethylsulfoxide Dimethyl Sulphoxide Phonophoresis on

Parameters of Oxidative Stress in Traumatized Muscle. *Ultrasound in Medicine and Biology*, 36(1): 44-50.

27. Chad, K. and Darryn, W. (2005). The antioxidant role of glutathione and N-acetyl-cysteine supplements and exercise-induced oxidative stress. *Journal of the International Society of Sports Nutrition*, 9;2(2):38-44.

28. Moffarts, B., Kirschvink, N., Art, T., Pincemail, J. and Lekeux, P. (2005). Effect of oral antioxidant supplementation on blood antioxidant status in trained thoroughbred horses. *The Veterinary Journal* 169: 65–74.

29. Ioannis, Z., Fiona, H., Eva, R., Laura, T., Sarah, S., Eveline, G., Nicolas, P., Pascal, S., Carmen, S. and Laia, M. (2020) Therapeutic potential of glutathione-enhancers in stress-related psychopathologies. *Neuroscience and Biobehavioral Reviews*, 114:134-155.