

Antioxidant Activities of Trévo on Some Oxidative Stress Markers And Micronutrients Status in Wistar Rats

Ogbolu D. Olusoga^{1*}, Alli O.A. Terry¹, Ayelagbe O. Gloria², Adesiyan A. Adekunle¹, Adebayo T. Olalekan², Oluremi S. Adeolu¹, Terigbade Khadijat¹, Adeyemo H. Babayemi¹.

¹Department of Biomedical Sciences, College of Health Sciences, Ladoke Akintola University of Technology, Ogbomoso, Nigeria

²Department of Chemical Pathology, College of Health Sciences, Ladoke Akintola University of Technology, Ogbomoso, Nigeria

Correspondence to: *Department of Biomedical Sciences, College of Health Sciences, Ladoke Akintola University of Technology, Ogbomoso, Nigeria.

Corresponding Author: Ogbolu David Olusoga

Abstract: Trévo, a food supplement has been claimed to have varying applications in the health and nutritional products. The purpose of this study was to investigate the ability of Trévo to protect against oxidative stress in vivo using animal models.

Study design and Methods: The study was carried out on 36 male Wistar rats which were randomly divided into six groups: Group 1 (Control) received normal feed and water only, Group 2 and 3 (Treatments) received Trévo at the dose of 0.9 ml /kg twice daily – Group 2 for 7 days, Group 3 for 14 days. Group 4 (Test) received 500 mg/kg acetaminophen daily for 10 days to achieve uremia. Group 5 (Test + Treatment) received acetaminophen and from 11th day receives Trévo supplement twice daily for 7 days. Group 6 (Test + Treatment) received acetaminophen and from 11th day received Trévo supplement twice daily for 14 days.

Results: The phytochemical screening of Trévo revealed the presence of alkaloids, tannin, phlobatannin, saponin, flavonoids, anthraquinones, terpenes, phenol, steroids and cardiac glycosides. Induction with paracetamol for Group 4 was significant ($P < 0.001$) for total antioxidant potential, alpha-tocopherol, Zinc (Zn), Iron (Fe), Selenium (Se), Cobalt (Co) and Manganese (Mn) levels. Similarly, a significant increase ($P < 0.001$) in plasma malondialdehyde (MDA), osmotic stress index (OSI), total plasma peroxide (TPP) levels were also obtained when compared with other groups.

Conclusion: These results clearly indicate beneficial effect of Trévo as a natural antioxidant hence, Trévo can be used to supplement diets to prevent and alleviate oxidative stress complications.

Key words: Trevo, Oxidative stress, Micronutrients, Phytochemical, Wistar rat.

Date of Submission: 03-01-2018

Date of acceptance: 16-01-2018

I. Introduction

Trévo is a known nutritional supplement that has physical and psychological benefits according to the manufacturer [1]. It is also believed to support weight management, growth management, cardiovascular system health, immune system health, healthy cholesterol, healthy blood sugar, digestive system health, proper pH levels, sports and overexertion recovery. It is thought to have cellular health benefit as it protects cells against detrimental effects of oxygen; including cancer-preventive properties [1]. Oxidative stress involves any condition in which oxidant metabolites (e.g., oxygen radicals) can exert their toxic effects due to increased production or altered cellular mechanisms of protection. Oxidative stress caused by oxygen toxicity may have adverse effects on virtually every organ of the body. The effects of oxidative stress can be evidenced by cellular accumulation of peroxides (e.g., lipid peroxides) or by-products, such as MDA, and by oxidized glutathione. Oxygen itself has a radical nature and can be called a di-radical, but it does not exert any major reactivity [2]. Oxidative stress (damage) created by free radicals reflects an imbalance between the systemic manifestation of reactive oxygen species and a biological system's ability to readily detoxify the reactive intermediates or to repair the resulting damage.

MDA is one of the fairly reactive metabolic products resulting from the effect of free oxygen radicals on tissues and from a series of reactions during lipid peroxidation [3]. The plasma MDA level is a sensitive indicator of lipid peroxidation and thus of oxidative stress [4]. Extensive lipid peroxidation leads to

disorganization of membrane by peroxidation of unsaturated fatty acids which may lead to a decrease in membrane fluidity and cell death [5].

Micronutrients are essential constituents of antioxidant system [6]. Certain vitamins (vitamins C, E and A) exert direct antioxidant effects while trace metals are integral parts of antioxidant enzymes. Zn, Cu and Mn are integral parts of superoxide dismutase, Se is an integral part of glutathione peroxidase and Fe is an integral part of catalase [6]. Vitamin C (ascorbic acid), a water soluble antioxidant is the most important free radical scavenger in extracellular fluids, trapping radicals in the aqueous phase and protects biomembranes from peroxidative damage [7]. Vitamin E (α -tocopherol), a fat soluble antioxidant is a powerful chain breaking antioxidant and resides primarily in biologic membranes, protecting membrane phospholipids from peroxidation [8].

Recently, more attention has been given to medicinal plants of therapeutic potentials as antioxidants in reducing free radical induced tissue injury. Therefore, the importance of searching for and exploiting natural antioxidants has increased greatly in present years [9]. Several studies have indicated that flavonoids are polyphenolic antioxidants naturally present in vegetables, fruits, and beverages such as tea and wine [10;11] which has also been claimed to be present in Trévo. Despite its very high cost, 11,000 – 12,000 Nigerian naira which is very expensive for an average Nigerian, the popularity of Trevo is on the increase vis-à-vis its use for treatment or prevention of varying diseases particularly the metabolic ones there is little or no data to support these claims. Hence, this study was designed to investigate the protective effect or otherwise of Trevo as a nutritive supplement on oxidative stress markers and micronutrients as parts of antioxidant system.

II. Materials And Methods

2.1 Study site

This study was carried out at the Department of Biomedical Science, College of Health Sciences, LAUTECH, Osogbo, Osun State.

2.2 Selection of Animals and Care

The study was conducted on thirty-six healthy adult male albino Wister rats that weighed between 100 and 160 g. They were acclimatized at the university animal house condition for two weeks prior to experimentation. Animals were housed six rats per cage and provided with standard feed and water extemporaneously. Animal care was provided according to the guiding principle for the care and use of animals [12]. Healthy and matured experimental male rats weighing between 100 and 160 g were used.

2.3 Grouping of Animals and Experimental Procedure

The rats were randomly divided into six equal groups as follows: Group 1 – Six rats were housed at room temperature ($25 \pm 3^{\circ}\text{C}$) and fed with normal feed and water. Group 2 – Six rats were placed in cage with normal diet, water and supplemented with Trévo at the dose of 0.9 ml per kg of body weight (Serving size: 1 fl. oz. / 30 ml, up to 2 ounces for an adult) per rat respectively, using an oral cannula for 7 days at 9.00 a.m. and 6.00 p.m. daily. Group 3 – Six rats were placed in cage with normal diet, water and supplemented with Trévo as in group 2 above for 14 days. Group 4 - Six animals were placed in cage with normal diet and water and were injected with acetaminophen at the concentration of 500 mg per kg of body weight per day for 10 days to achieve uremia [13]. Group 5 – Six animals were placed in cage and were injected with acetaminophen to achieve uremia and co-administered with Trévo at the dose of 0.9 ml per kg body weight per rat, respectively, using an oral cannula for 7 days at twice daily 9.00 a. m. and 6.00 p. m. Group 6 – Six animals were placed in cage and were injected with acetaminophen to achieve uremia and co-administered with Trévo as in group 5 for 14 days.

2.4 Preparation of Experimental Dose of Acetaminophen (Paracetamol)

Paracetamol injection (NAFDAC registration number 04-0411) was obtained at a pharmaceutical store in LAUTECH hospital area, Osogbo. Each ampoule contained 300 mg/2 ml of paracetamol solution (i.e. 150 mg/ml). A dose of 500 mg/kg of paracetamol for 10 days known to cause uremia [13] was calculated from the stock solution for each animal thus;

$$\text{Administered volume} = \frac{\text{Normal dose}}{1000} \times \frac{\text{Body weight}}{\text{Stock solution}}$$

2.5 Calculation of Administered Dose of Trévo

Serving Size: 1 Fluid Ounces (29.57ml), up to 2 ounces for an adult [1].

If 1 fl. oz. = 30 ml

2 oz. = $30 \times 2 = 60$ ml

Then, 60 ml = 70 kg (Weight of an average adult)

Therefore, 1 kg = 60/70

= 0.85

= 0.9 ml/kg of Trévo

2.6 Sample Collection

Venous blood samples were collected at different stages from all the animal groups for biochemical analysis as follows: Baseline samples were collected from all the groups; another set of samples were collected on 11th day of induction of the test groups from half of the members of group 4 prior to Trévo supplementation of groups 2, 3, 5 and 6; samples were also collected from members of groups 2 and 5 on 7th day of the Trévo supplementation of the two groups; lastly samples were collected on the 25th day of the experiment from the rest of the members of the groups.

2.7 Phytochemical Screening

Phytochemical analysis of Trévo was done using standard procedures as described by Association of Official Analytical Chemists [14].

2.8 Estimation of Malondialdehyde (MDA)

Level of lipid peroxidation was determined by measuring the formation of MDA using the method of Varshney and Kale [15]. This procedure was based on the fact that MDA produced from the peroxidation of membrane fatty acid reacts with the chromogenic reagent 2-thiobarbituric acid (TBA) under acidic conditions to yield a pink-coloured complex measured spectrophotometrically at 532 nm. 1, 1, 3, 3-tetramethoxypropane was used as standard.

2.9 Estimation of Total Antioxidant Potential (TAP)

TAP was determined using the ferric reducing/ antioxidant power (FRAP) assay [16;17]; 1.5 ml of working pre-warmed 37°C FRAP reagent (300 mM acetate buffer - pH 3.6, 10 mM 2,4,6- tripyridyl-s-triazine in 40 mM HCl and 20 mM FeCl₃ at ratio 10:1:1) was vortex mixed with 50 µl of test sample and standards. Absorbance was read at 593 nm against a reagent blank. The result was reported as µmol Trolox equiv/L.

2.10 Estimation of Total Plasma Peroxidase (TPP)

Determination of TPP levels made use of the reaction of ferrous-butylated hydroxytoluene-xylene orange complex (FOX-2 reagent) with plasma hydrogen peroxide, which yields a colour complex that was measured spectrophotometrically at 560 nm. H₂O₂ was used as standard. 1.8 ml of FOX-2 reagent was mixed with 200 µl of plasma. This was incubated at room temperature for 30 min. 100 µM H₂O₂ was used as standard. The mixture was centrifuged and the supernatant separated for reading at 560 nm[17].

2.11 Determination of oxidative stress index (OSI)

OSI, an indicator of the degree of oxidative stress, is the percent ratio of the TPP to the TAP values [17].

2.12 Determination of plasma level of vitamin E

The level of vitamin E was determined in the plasma level by using the method of Baker [18] which is based on the principle that vitamin E extracted in xylene is made to react with alpha, alpha-dipyridyl. The product produces a reddish color with ferric chloride, which was read at 520 nm.

2.13 Determination of plasma levels of trace metals

Plasma levels of trace elements were determined with flame atomic absorption spectrophotometer (AAS) using a direct method as described by Kaneko[19]. The method is based on the principle that atoms of the element when aspirated into AAS vaporized and absorbed light of the same wavelength as that emitted by the element when in the excited state. The wavelengths used for each of the trace elements are Fe; 248.3, Zn; 213, Se; 196, Mn; 279.40, Co; 395.

2.14 Statistical Analysis

The Data obtained were analyzed with statistical package for social sciences (SPSS) version 17. The data were expressed in mean ± SD. The difference between the means was analyzed by one-way analysis of variance (ANOVA). *P*-value <0.001 was considered statistically significant.

III. Results

Table 1 showed the phytochemical constituents of Trévo analysed qualitatively. The levels of alkaloids, tannin, flavonoids, saponin and phenol were appreciably high, while the concentrations of phlobatannin, steroids, terpenes and cardiac glycosides were moderate with trace amount of anthraquinones, while complete absence of cardenolides and chalcones was found.

The mean plasma Malondialdehyde (MDA), total plasma peroxide (TPP) and oxidative stress index (OSI) were increased in the test group while total antioxidant potential (TAP) and α -tocopherol (vitamin E) levels were decreased when compared with other groups. The mean of the markers differed between the groups with marked increase in plasma MDA, TPP and OSI of the test groups and marked decrease in plasma TAP and

α -tocopherol of the test groups. When group 4 MDA, OSI and TPP were compared to groups 1, 2, 3, 5 and 6, it showed a significance change ($P < 0.001$) in osmotic stress index indicating antioxidative effect of Trévo (Table 2). All the trace metals, Mn; Fe; Co; Zn; Se in the test group 4 were significantly decreased ($P < 0.001$) compared to the treatment groups (2, 3, 5, 6) including control group. Also, the plasma trace elements levels differ in all groups, there were decrease in the plasma trace elements levels of the test groups compared with other groups with some showing significant difference ($P < 0.001$) (Table 3).

IV. Discussion

Plants have been the basis of traditional medicines throughout the world for thousands of years and continue to provide new remedies to human kind; a great deal of effort has therefore focused on natural antioxidants from plants as food supplements [20]. In spite of tremendous development in the field of allopathic medicines during the 20th century, plants still remain one of the major sources of drugs in modern as well as in traditional system of medicine. Medicinal plants are source of certain bioactive molecules which act as antioxidants and antimicrobial agents [20]. Trévo is a dietary supplement made from plants with physical and psychological benefits according to the manufacturer [1]. The phytochemicals revealed that phenolic compounds are found in appreciable amount and fall into several categories such as simple phenolics, phenolic acids (derivatives of cinnamic and benzoic acids), coumarins, flavonoids, stilbenes, tannins and lignans, nitrogen compounds such as alkaloids also contain antioxidant activity [21;22]. Dietary flavonoids represent a diverse range of polyphenolic compounds that occur naturally in plant foods. Flavonoids from food have been reported to be potentially involved in decreasing oxidative stress by modulating endothelial mechanisms responsible for atherosclerosis [23]. Plants are generally believed to be rich in wide variety of secondary metabolites such as alkaloids, flavonoids, terpenes, saponins which have received increase attention as useful nutraceuticals in the management of oxidative stress diseases [24].

The increase in the MDA levels of the paracetamol induced rats in this regard is in agreement with the findings of Pradhan *et al.* [13] who found higher MDA levels in paracetamol induced rats when compared with control groups. There was a reduction in MDA levels in group 5 (Test + one week treatment) and group 6 (Test + two weeks treatment) but this was not statistically significant ($P > 0.05$). The reduction of MDA levels in group treated with Trévo may be due to the presence of phytochemicals like phenols as stated by Khalil *et al.* [25] that plant materials containing phenolics retard oxidative modification of lipids and thereby improving quality and nutritional value of food. Our data showed that paracetamol induced oxidative stress affects plasma antioxidants status as well as causes membrane lipid peroxidation (MDA). MDA is a highly toxic by-product formed by oxidation derived from free lipid radicals, and there is considerably raised concentrations in oxidative stress.

Certain vitamins (vitamins C, E and A) exert direct antioxidant effects while trace metals are integral parts of antioxidant enzymes. Zn, Cu and Mn are integral parts of superoxide dismutase. Se is an integral part of glutathione peroxidase and Fe is also an integral part of catalase [6]. The TAP is therefore an index of all classes of antioxidants. For TAP, positive control group 4 was compared to groups 1, 2, 3, 5 and 6; this showed a significant decrease at groups 1, 2 and 3 but not significant with groups 5 and 6 meanwhile, group 2 showed a significant decrease when compared with group 3.

TPP showed a significant increase in the positive control (group 4) at groups 2 and 3 ($P < 0.001$) but not significant ($P > 0.05$) with groups 1, 5 and 6. OSI also showed a significant increase in the positive control (group 4) when compared with groups 1, 2, 3, 5 and 6. α -tocopherol showed a significant decrease in group 4 when compared with groups 2 and 3 whereas not significant with groups 1, 5 and 6, this may be as a result of lipid peroxidation without supplementation that occurs in group 4 [26;27], this shows that α -tocopherol is the most important lipid-soluble antioxidant and it protects membranes from oxidation by reacting with lipid radicals produced in the lipid peroxidation chain reaction.

The plasma trace metals (Zn, Fe, Co, Se, Mn) in test group 4 showed a significant decrease when compared with groups 1, 2, 3, although the difference was not statistically significant ($P > 0.05$) when compared with groups 5 and 6. Manganese and zinc potentiate the antioxidant activities of superoxide dismutase, which may contribute to high levels of total antioxidants observed in this study. Mn plays an important role in a number of physiologic processes as a constituent of some enzymes and as an activator of different enzymes. These Mn activated enzymes play important roles in maintenance of plasma antioxidant system [28]. Therefore, Mn deficiency in diseases could predispose to oxidative stress.

Hall *et al.* [7] and Galasko *et al.* [29] reported that micronutrients levels were markedly decreased in the setting of oxidative stress complications hence the need for further supplementation. Trévo shows significant antioxidant activity on paracetamol induced oxidative stress in Wistar rats. It was also found to stabilize some plasma trace elements like Se, Fe, Zn, Mn and Vitamin E level. However, this study strongly recommends comprehensive clinical trials to confirm the detailed antioxidant constituents of Trévo both qualitatively and quantitatively and also its recommendation for the treatment of diseases associated with oxidative stress especially the metabolic ones.

Acknowledgement

The authors would like to thank the staff of Biomedical Science department particularly the Medical Laboratory Scientists and the staff at the Animal House unit of the department for their unflinching support.

Conflicts of Interest Statement

Authors have no conflicts of interest to declare regarding this study.

References

- [1]. Stevens MA: Trévo – The power of wellness. Trevo web site; 2015 [updated 4 June, 2015; Cited 14 Dec, 2015]; Available from: www.trevocorporate.com/what-is-trevo/index.html.
- [2]. Ceconi C, Boraso A, Cargnoni A, Ferrari R: Clinical manifestations of oxidative stress. *Archives of Biochem & Biophys*, 420(2), 2003, 217-221.
- [3]. Weinberger B, Anwar M, Henien S: Association of lipid peroxidation with antenatal beta-metazone and oxygen radical disorders in preterm infants. *Biol Neonate*, 85 (2), 2004, 121-127.
- [4]. Kirimi E, Peker E, Tuncer O: Increased serum malondialdehyde level in neonates with hypoxic ischemic encephalopathy: Prediction of diseases severity. *J Int Med Res*, 38 (1), 2010, 220-226.
- [5]. Onyeka C, Aligwekwe A, Nwakanma A: Effect of ethanolic root bark extract of *Chrysophyllum albidum* on serum SOD, catalase and MDA in rats. *Int J Pha Sci Res*, 3(3), 2012, 347-351.
- [6]. Ruffin MT, Rock CL: Do antioxidants still have a role in the prevention of human cancer? *Curr Oncol Rep*, 3(4), 2001, 306-313.
- [7]. Hall ED, Vaishnay RA, Mustafa AG: Antioxidant therapies for traumatic brain injury. *J Neurother*, 7(1), 2010, 51-61.
- [8]. Hfaiedh N, Murat JC, Elfeki A: A combination of ascorbic acid and a-tocopherol or a combination of Mg and Zn are both able to reduce the adverse effects of lindane-poisoning on rat brain and liver. *J Trace Elem Me Biol*, 26(4), 2012, 273-278.
- [9]. Erdemoglu N, Turan NN, Cahoco I: Antioxidant activities of some lamiaceae plant extracts. *Phytother Res*, 20, 2006, 9-13.
- [10]. Renaud S, De Lorgeril M: Wine, alcohol, platelets, and the French paradox for coronary heart disease. *Lancet*, 339, 1992, 1523-1526.
- [11]. Hertog MGL, Feskens EJM, Kromhout D: Dietary antioxidant flavonoids and risk of coronary heart disease: The Zutphen elderly study. *Lancet*, 342, 1993, 1001-1011.
- [12]. Gonder JC, Laber K: A renewed look at laboratory rodent housing and management. *ILAR J*, 48, 2007, 29-36.
- [13]. Pradhan S, Mandal S, Roy S: Attenuation of uremia by orally feeding alpha-lipoic on acetaminophen induced uremic rats. *Saudi pharm J*, 21(2), 2012, 187-192.
- [14]. Association of Official Analytical Chemists: Official Methods of Analysis (14th Ed. Washington DC, USA, 1984; p 522–533).
- [15]. Varshney R, Kale RK: Effect of calmodulin antagonist on radiation-induced lipid peroxidation in microsomes. *Int J Rad Biol*, 58, 1990, 733-743.
- [16]. Benzie IF, Strain JJ: The ferric reducing ability of plasma (FRAP) as a measure of antioxidant power (The FRAP) assay. *Annal. Biochem*, 239, 1996, 70-76.
- [17]. Harma M, Enel O: Increased oxidative stress in patients with hydatiform mole. *Swiss Med Wkly*, 133, 2003, 563-566.
- [18]. Baker F: Clinical vitaminology (New York Willey 1968; p. 172).
- [19]. Kaneko JJ: Clinical biochemistry of animals (Acad. Press. Inc. New York 1999; p932).
- [20]. Krishnaiah D, Sarbatly R, Nithyanandam R: A review of the antioxidant potential of medicinal plant species. *Food Bioprod Pro*, 89 (3), 2011, 217-233.
- [21]. Velioglu YS, Mazza G, Gao L: Antioxidant activity and total phenolics in selected fruits, vegetables and grain products. *J Agric Food Chem*, 46(10), 1998, 4113-4117.
- [22]. Kinjo J, Hitoshi M, Tsuchihashi R: Protective effects of naturally occurring flavonoids and miscellaneous phenolic compounds. *J Nat Med*, 60, 2006, 36-41.
- [23]. Grassi D, Desideri G, Ferri C: Flavonoids: Antioxidants against atherosclerosis. *J Nut*, 2, 2010, 889-902.
- [24]. Wan J, Diaz-Sanchez D: Antioxidant enzyme induction: A new protective approach against the adverse effects of diesel exhaust particles. *Inhal Toxicol*, 19, 2007, 177-182.
- [25]. Khalil MY, Moustafa AA, Naguib NY: Growth, phenolic compounds and antioxidant activity of some medicinal plants grown under organic farming condition. *W J Agric Sci*, 3(4), 2007, 451-457.
- [26]. Herrera E, Barbas C: Vitamin E: action, metabolism and perspectives. *J Physiol Biochem*, 57(2), 2001, 43-56.
- [27]. Packer L, Weber SU, Rimbach G: Molecular aspects of alpha-tocotrienol antioxidant action, *J Nutr*, 31(2), 2001, 369-373.
- [28]. Nicolof GK, Mutaftachiev V, Petrova C: Serum Mn in children with diabetes mellitus type, *Diabetologia Critical*, 3, 2004, 47-51.
- [29]. Galasko DR, Peskind E, Christopher MC, Joseph FQ, John MR, Gregory AJ et al: Antioxidants for Alzheimer disease: A randomized clinical trial with cerebrospinal fluid biomarker. *Arch Neurol*, 85, 2012, 1-6.

Table 1. Qualitative amount of phytochemical constituents of Trévo

| Phytochemical | Observation |
|--------------------|-------------|
| Alkaloids | +++ |
| Tannin | +++ |
| Phlobatannin | ++ |
| Saponin | +++ |
| Flavonoids | +++ |
| Anthraquinones | + |
| Steroids | ++ |
| Terpenes | ++ |
| Cardenolides | - |
| Phenol | +++ |
| Chalcones | - |
| Cardiac glycosides | ++ |

+: Trace; ++: Moderate; +++: Appreciable; -: Absent

Table 2. Activity of Trévo on oxidative stress markers

| Group | MDA (nmol/ml) | TAP (umolTrolox Equi/L) | TPP(umolH ₂ O ₂ /L) | OS I (%) | α-TOCO (mg/L) |
|--|---------------|-------------------------|---|-----------|---------------|
| Panel 1: Effect of Trévo on oxidative stress markers | | | | | |
| 1 | 2.20±0.08 | 500.00±66.33 | 4.85±0.38 | 0.99±0.22 | 4.40±1.49 |
| 2 | 2.03±0.06 | 543.33±46.19 | 3.50 ±0.66 | 0.65±0.14 | 6.87±0.23 |
| 3 | 1.80±0.14 | 1127.50 ±17.68 | 2.70±0.14 | 0.24±0.01 | 8.60±2.83 |
| 4 | 3.05±0.25 | 293.33±52.52 | 5.67±0.31 | 1.98±0.46 | 3.33±0.31 |
| 5 | 3.00±0.51 | 468.33±114.05 | 4.87±0.31 | 1.08±0.27 | 4.33±0.31 |
| 6 | 0.90±0.14 | 485.00±35.36 | 4.90±0.14 | 1.02±0.11 | 5.50±1.27 |
| Panel 2: Comparison of mean of oxidative stress parameters of test group with other groups | | | | | |
| Pa | 0.00* | 0.01* | 0.27 | 0.00* | 0.88 |
| Pb | 0.00* | 0.01* | 0.00* | 0.00* | 0.27 |
| Pc | 0.00* | 0.00* | 0.00* | 0.00* | 0.00* |
| Pd | 0.21 | 0.06 | 0.35 | 0.01* | 0.93 |
| Pe | 0.21 | 0.07 | 0.52 | 0.01* | 0.43 |
| Pf | 0.95 | 0.01* | 0.47 | 0.47 | 0.59 |

P-value < 0.001 considered significant when compared with the test group (Group 4)

Groups, 1: Control – Feed only; 2: Treatment – Feed + Trévo (0.9 ml/kg) for one week; 3: Treatment – Feed + Trévo (0.9 ml/kg) for two weeks; 4: Test – Feed + Paracetamol (500 mg/kg for 10 days) (positive control); 5: Test – Feed + Paracetamol (500 mg/kg for 10 days) + Trévo (0.9 ml/kg) for one week treatment; 6: Test – Feed + Paracetamol (500 mg/kg for 10 days) + Trévo (0.9 ml/kg) for two weeks treatment. Pa: Significance of difference between group 4 and 1; Pb: Significance of difference between group 4 and 2; Pc: Significance of difference between group 4 and 3; Pd: Significance of difference between group 4 and 5; Pe: Significance of difference between group 4 and 6; Pf: Significance of difference between group 2 and 3; *: Shows values significant.

Table 3. Activity of Trévo on micronutrients status (Mean ± SD)

| Group | Zn (mg/L) | Fe (mg/L) | Co (mg/L) | Se (mg/L) | Mn (mg/L) |
|---|-------------|-------------|-------------|-------------|-----------|
| Panel 1: Effect of Trévo on micronutrients status | | | | | |
| 1 | 0.08 ± 0.00 | 0.09 ± 0.00 | 0.02 ± 0.00 | 0.00 ± 0.00 | 0.02±0.00 |
| 2 | 0.71 ± 0.71 | 0.48 ± 0.45 | 0.03 ± 0.00 | 0.01 ± 0.00 | 0.01±0.00 |
| 3 | 0.60 ± 0.60 | 0.42 ± 0.08 | 0.02 ± 0.00 | 0.24 ±0.01 | 0.03±0.00 |
| 4 | 0.07 ± 0.01 | 0.03 ± 0.00 | 0.02 ± 0.00 | 0.04±0.00 | 0.01±0.00 |
| 5 | 0.21 ± 0.16 | 0.03 ± 0.00 | 0.02 ± 0.00 | 0.07 ± 0.00 | 0.02±0.00 |
| 6 | 0.30 ± 0.00 | 0.00±35.36 | 0.02 ± 0.00 | 1.02±0.00 | 0.05±0.00 |
| Panel 2: Comparison of mean of trace elements of test group with other groups | | | | | |
| Pa | 0.00* | 0.01* | 0.54 | 0.06 | 0.00* |
| Pb | 0.00* | 0.00* | 0.05* | 0.01* | 0.01* |
| Pc | 0.00* | 0.00* | 0.04* | 0.00* | 0.00* |
| Pd | 0.01* | 0.59 | 0.59 | 0.03* | 0.09 |
| Pe | 0.21 | 0.66 | 0.59 | 0.01* | 0.59 |
| Pf | 0.95 | 0.99 | 0.99 | 0.99 | 0.99 |

P-value < 0.001 considered significant when compared with the test group (Group 4)

Groups, 1: Control – Feed only; 2: Treatment – Feed + Trévo (0.9 ml/kg) for one week; 3: Treatment – Feed + Trévo (0.9 ml/kg) for two weeks; 4: Test – Feed + Paracetamol (500 mg/kg for 10 days) (positive control); 5: Test – Feed + Paracetamol (500 mg/kg for 10 days) + Trévo (0.9 ml/kg) for one week treatment; 6: Test – Feed + Paracetamol (500 mg/kg for 10 days) + Trévo (0.9 ml/kg) for two weeks treatment. Pa: Significance of difference between group 4 and 1; Pb: Significance of difference between group 4 and 2; Pc: Significance of difference between group 4 and 3; Pd: Significance of difference between group 4 and 5; Pe: Significance of difference between group 4 and 6; Pf: Significance of difference between group 2 and 3; *: Shows values significant.

Ogbolu D. Olusoga "Antioxidant Activities of Trévo on Some Oxidative Stress Markers And Micronutrients Status in Wistar Rats." IOSR Journal of Pharmacy and Biological Sciences (IOSR-JPBS) 13.1 (2018): 40-45.