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Research Article

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Effect of Trévo Dietary Supplement on Caffeine Induced Oxidative Stress in Albino Rat Models

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Abstract This study was aimed at investigating the effect of Trévo dietary supplement (TDS) in caffeine induced oxidative stress in albino rat models. Thirty healthy male albino rats of 12 weeks old were divided into five groups with six rats in each group using a completely randomized design (CRD). The rats were treated with combinations of caffeine and TDS orally via oral gavage. The treatment lasted for 65 days. Results of the oxidative stress markers showed a statistically significant ($P<0.05$) decrease in the plasma level of superoxide dismutase (SOD), catalase activity (CAT) and glutathione peroxidase (GPx) in caffeine treated groups when compared with the control group; also with an increase in the level of malondialdehyde (MDA) in caffeine treated rats. However, TDS attenuated the caffeine-induced oxidative stress in the albino rat models in a dose dependent manner. This study therefore, reveals that TDS is effective in attenuating caffeine-induced oxidative stress in albino rat models.

Keywords Caffeine, Trévodietary supplement, oxidative stress, malondialdehyde

Introduction

Caffeine (1, 3, 7-trimethylxanthine) is present in energy drinks, drugs, several food and beverage products, such as coffee, tea and carbonated beverages [1-2]. It is widely consumed, primarily for its stimulating effect on the central nervous system. Caffeine and other methylxanthines are used in clinical medicines as diuretics, analgesics and muscle relaxants, and can also aid in the treatment of brain disorders such as headaches and Parkinson's disease [3-4]. The cortical stimulation produced by caffeine results in mental alertness and reduces drowsiness and fatigue. In contrasts, high doses of caffeine induce nervousness, insomnia, tremors and other effects [5].

Oxidative stress depicts the presence of free radicals and reactive oxygen species (ROS) formed under normal physiological conditions but become harmful and deleterious when there are not eliminated by antioxidant systems [6]. In general, ROS are able to react with diverse cellular components (e.g. DNA, carbohydrates, proteins and lipids) in a destructive way, therefore, the balance between ROS and antioxidant species play an important role in preventing oxidative stress [7].

Some of the effects of caffeine may favour the production of free radicals and lead to a subsequent increase in lipid peroxidation by increasing oxidative stress [6, 8-9]. Also, mitochondrial pathways that oxidize energy substrates and carry out respiration, produces significant amounts of ROS, which leak out of the mitochondria and cause substantial damage to various cellular components. Thus, enhanced mitochondrial activity is expected to enlarge the ROS pool and, in turn, contribute to oxidative stress [8, 10]. Oxidative stress has also been reported to cause significant damage to biological molecules such as lipid peroxidation, DNA damage and decline in sperm quality [11-14].

Trévo dietary supplement (TDS) is a multi-herbal health drink containing 174 ingredients (including 13 essential vitamins and 14 minerals as well as 58 plant and sea trace minerals, 20 amino acids, plant based essential fatty acids, antioxidants, digestive enzymes and coenzymes derived from graviola, 5 green super foods, 18 other garden and sea vegetables, 24 exotic and garden fruits) [15].

TDS is also said to have the potentials to promote good health, increase energy and maintain effective cardiovascular system, amongst others [15]. There is dearth of published literature on the uses and health benefits of TDS. However, many medicinal plants with antioxidant properties have been shown to confer ameliorating or protective effects from toxicities induced by substances such as caffeine [16-22].

In view of the increasing intake of caffeine and its abuse which is a reoccurring habit that may cause toxicities, oxidative stress and cellular damage. This study is designed to ascertain the attenuating effect of TDS on caffeine induced oxidative stress in albino rats as mammalian model, due to its rich cocktail of herbs and other ingredients.

Materials and Methods

Treatments and other Chemicals

Caffeine was obtained from Sigma-Aldrich (St. Louis, MO, USA), while Trévo dietary supplement (TDS) was obtained from a registered distributor in Calabar, Cross River State, Nigeria. TDS is manufactured by United International Lab LLC, TX 75244, USA for TRÉVO L LC ^{TM}, OK 73107, USA, under the trade name TRÉVO. All other chemicals used in this study were of analytical grade.

Experimental Animals

Thirty healthy male albino rats of 12 weeks old; with average body weight of 176.5g were obtained from the animal house of the Department of Genetics and Biotechnology, University of Calabar, Calabar for this study. The rats were housed in well ventilated wire mesh cages under standard laboratory conditions. They were allowed free access to water and pelleted commercial feed throughout the period of the experiment. Generally, the study was conducted in accordance with the recommendations from the declarations of Helsinki on guiding principles in care and use of animals and the local ethical committee.

Experimental Design and Procedure

The thirty rats were divided into five groups of six rats each using a completely randomized design. The animals were acclimatized for one week before the commencement of the treatment. The daily treatments were given orally via oral gavage which lasted for 65 days and the protocol is shown in Table 1. The rats were sacrificed under chloroform anaesthesia 24 hours after the last treatment. Blood samples were collected through cardiac puncture and the serum was used for the analyses of the following oxidative stress markers: superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GPx), and malondialdehyde (MDA).

Table 1: Protocol for daily treatment of experimental animals

Treatment groups	Description of treatment
Control	No caffeine and no Trévo dietary supplement (TDS)
C	Caffeine, 200mg kg^{-1} BW only via oral gavage
T_1	Trévo dietary supplement (TDS), 1mL kg^{-1} BW only via oral gavage
C+T_1	Caffeine, 200mg kg^{-1} BW and 10-12 hours after TDS, 1mL kg^{-1} BW both orally via oral gavage
C+T_2	Caffeine, 200mg kg^{-1} BW and 10-12 hours after TDS, 2mL kg^{-1} BW both orally via oral gavage

Analyses of Oxidative Stress Markers

Superoxide Dismutase

Superoxide dismutase (SOD) activity was determined according to the method developed by Fridovich [23] which is based on the inhibition of superoxide radicals' reaction with phenyl tetrazolium chloride.

Catalase

Catalase (CAT) activity was measured according to the method of Aebi [24]. The activity of catalase was based on the disappearance of hydrogen peroxide.

Glutathione Peroxidase

Glutathione peroxidase (GPx) activity was assessed by measuring the rate of NADPH oxidation in a spectrophotometer at 340 nm and expressed as international units/ml [25].

Malondialdehyde

Malondialdehyde (MDA) levels were measured by the method developed by Ohkawa*et al.* [26] based on the measurement of absorbance of thiobabituric acid.

Statistical analysis

Data fromthe oxidative stress markers (superoxide dismutase, catalase, glutathione peroxidase and malondialdehyde) were subjected to analyses of variance (ANOVA) test for significant difference. Statistical significance were considered if $p<0.05$ while least significant difference (LSD) test was used to separate the means.

Results

Superoxide Dismutase (SOD)

Figure 1 shows that the concentration of SOD was significantly ($P<0.05$) reduced in caffeine group when compared to the control. Treated animals in the caffeine group had the lowest value of 88.17IU/ml. The concentration of SOD in caffeine group was reduced by 50.56% when compared with the control group. The values were significantly increased in $C+T_1$ and $C+T_2$ groups (141.67 and 158.50IU/ml, respectively) indicating a dose-dependent attenuating effect. The highest concentration of SOD was recorded in the T_1, group (196.17IU/ml) while the control had 178.33IU/ml.

Glutathione Peroxidase (GPx)

Caffeine caused a significant ($P<0.05$) reduction in the concentration of GPx in the treated animals with the lowest value observed in the caffeine group (44.92 IU/ml). The concentration of GPx in caffeine group was reduced by 42.72% when compared with the control group. The effect of caffeine was attenuated in the $C+T_1$ and $C+T_2$ groups with mean values of 61.37 and 71.20 IU/ml, respectively. T_1 group had the highest concentration of GPx being 93.37 IU/ml followed by the control (78.42 IU/ml) as shown in Figure 1.

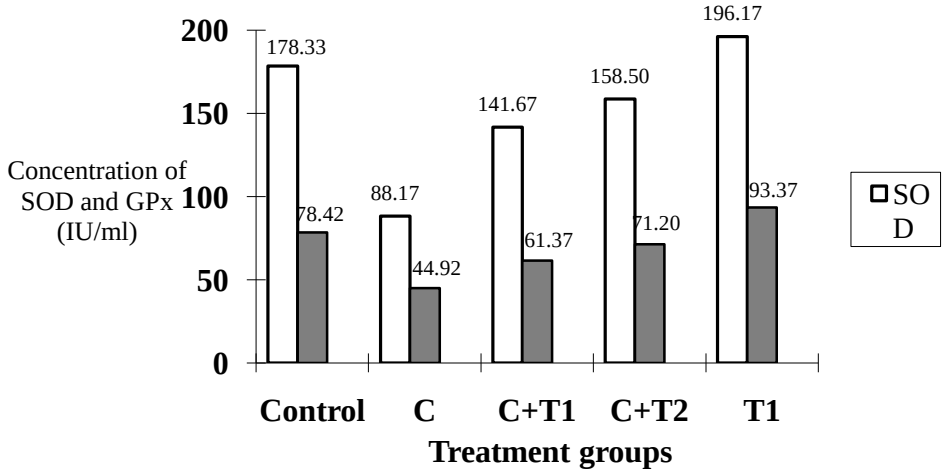

Figure 1: Effect of Trévo dietary supplement on superoxide dismutase (SOD) and glutathione peroxidase (GPx) in rats

Catalase (CAT)

Catalase concentrations were significantly (P<0.05) reduced in the caffeine treated groups when compared to the control (Figure 2). The concentration of catalase in caffeine group was reduced by 48.89% when compared with the control group. Caffeine, C+T_1 and C+T_2 had mean values of 0.68, 0.88 and 0.95 IU/ml, respectively while the control group had 1.15 IU/ml. The highest concentration of catalase was obtained in the T_1 group.

Malondialdehyde (MDA)

MDA was significantly increased (P<0.05) by caffeine treatment. The concentration of MDA in caffeine group was increased by 65.73% when compared with the control group. Caffeine group had a concentration of 2.95nmol/ml, followed by C+T_1 (2.80nmol/ml) and C+T_2 (2.02nmol/ml) indication of dose-dependent attenuating effect of TDS. The control and T_1 groups had 1.78 and 1.35nmol/ml, respectively (Figure 2).

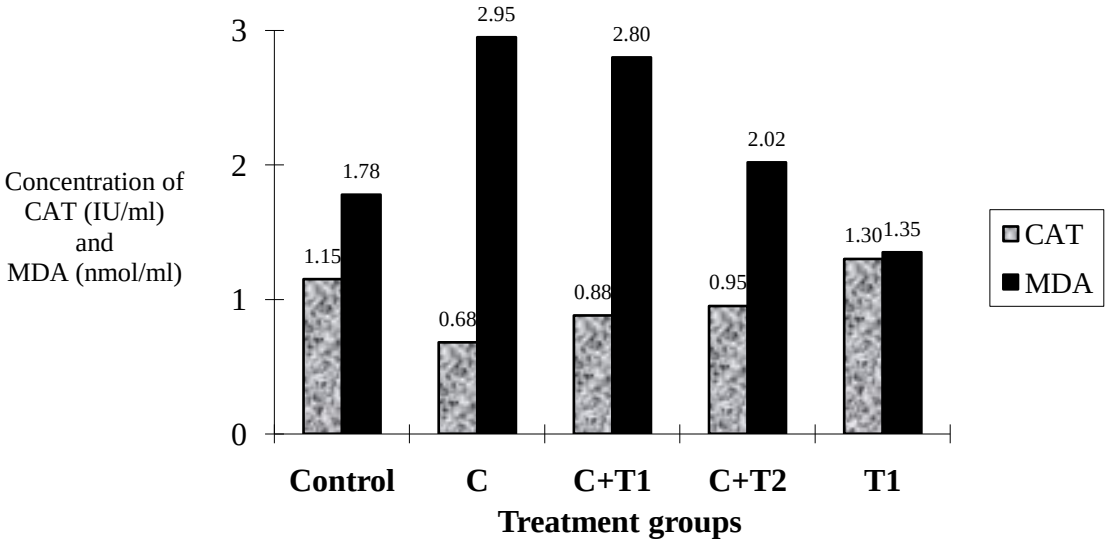

Figure 2: Effect of Trévo dietary supplement on catalase (CAT) and malondialdehyde (MDA) in rats

Discussion

Result obtained in this study revealed that caffeine significantly affected the oxidative stress markers assayed. The concentration of the antioxidants (SOD, GPx and CAT) significantly (P<0.05) reduced in caffeine treated animals which agrees with the findings of Hatice *et al.* [27]. These antioxidants converted free radicals or reactive oxygen intermediates to non-radical and harmless products [28]. Previous studies suggest that SOD, CAT and GPx are the major scavengers of harmful ROS in organs [29]. Kermal *et al.* [30] showed that attack on the DNA by ROS produce base free sites, deletion, frame-shift mutations, DNA cross links and chromosomal rearrangements. In a related study, it was observed that neurological impairment is intrinsically linked to ROS-triggered neuronal apoptosis [7]. The significant dose-dependent reductions in SOD, CAT and GPx concentrations by caffeine treatment indicates a decrease in antioxidant defense system, increased free radical activities and consequently resulting in oxidative stress. However, TDS attenuated the caffeine induced oxidative stress by increasing the concentrations of SOD, CAT and GPx in C+T1 and C+T2 groups (Figures 1and 2).

On the other hand, the concentration of MDA significantly increased in caffeine treated groups which indicate an increased lipid peroxidation activity. Lipid peroxidation is an important biological consequence of oxidative cellular damage; hence the increased concentration of MDA reflects oxidative stress [6, 8]. The increase in the concentration of MDA in caffeine treated animals is similar to the findings of Dianzani*et al.*[31]. Increase in lipid peroxidation also inhibits the activity of antioxidative enzymes such as SOD, GPx and CAT as well as total antioxidant status [32].

The attenuating potential of TDS was also observed as it reduced the concentration of MDA from 2.95 nmol/ml in caffeine group to 2.02 nmol/ml in C+T2 group (Figure 2). These attenuating effects can be attributed to the different antioxidants, vitamins and other phytonutrients present in TDS. Dietary antioxidants have been reported to play a major role in the maintenance of oxidative balance with antioxidants such as bioflavonoids; considered as an efficacious antioxidant which is widely distributed in fruits and leafy vegetables [16-22, 33]. This therefore implies that the various constituents of the TDS must have played a major role in scavenging free radicals that can accumulate to cause lipid peroxidation and oxidative damage to proteins and DNA [34] as seen in the caffeine treated animals.

Conclusion

The results of the present study provide substantial evidence that Trévo dietary supplement (TDS) is effective in attenuating caffeine induced oxidative stress in albino rat models; and also in a dose dependent manner.

References

1. Ekaluo, U. B., Udokpoh, A. E., Udofia, U. U. & Ajang, R. O. Comparative toxicity of five commonly used analgesics on sperm count and sperm head abnormalities. *Global Journal of Pure and Applied Sciences*, 2005, 11: 81-84.
2. Ekaluo, U. B., Ikpeme, E. V. & Udokpoh, A. E. Sperm head abnormality and mutagenic effects of aspirin, paracetamol and Caffeine containing analgesics in rats. *The Internet Journal of Toxicology*, 2009, 7: 1-9.
3. Graham, I. Caffeine and exercise metabolism, endurance and performance. *Sport Medicine*, 2001, 31: 785-807.
4. Kolayli, S., Osak, M., Kucuk, M. &Abbasoglu, R. Does caffeine bind to metal ions? *Food Chemistry*, 2004, 84: 383-388.
5. Fredholm, B. B., Bahig, K., Holmen, J., Nehlig, A. & Zvartau, E. E. Actions of caffeine in the brain with special references to factors that contribute to its widespread use. *Pharmacological Review*, 1999, 51:83-133.
6. Fang, Y. Z., Yang, S. & Wu, G. Free radical, antioxidant and nutrition. *Nutrition*, 2002, 11:872-890.
7. Halliwel, B. & Gutteridge, J. M. C. *Free radicals in Biology and Medicine*, 2005, Oxford, Oxford University Press, 2005.
8. Vistisen, K., Poulsen, H. & Loft, S. Foreign compound metabolism capacity in man measured from metabolites of dietary caffeine. *Carcinogenesis*, 1992, 13:1561-1568.
9. Gutteridge, J. M. C. & Halliwel, B. *Antioxidants in nutrition, health and disease.* Oxford, Oxford University press, 1994.
10. Beckman, K. B. & Ames, B. N. The free radical theory of aging matures. *Physiological Review*, 1998, 78: 547-581.
11. Makker, K., Agarwal, A. & Sharma, R. Oxidative stress and male infertility. Indian *Journal of Medical Research*, 2009, 129: 357-367.
12. duPlessis, S. S., McAllister, D. A., Luu, A., Savia, J., Agarwal, A. & Lampiao, F. Effects of H_2O_2 exposure on human sperm motility parameters, reactive oxygen species levels and nitric oxide levels. *Andrologia*, 2010, 42:206-210.
13. Zribi, N., Chakroun, N. F., Elleuch, H., Abdallah, F. B., Ben-Hamida, A. S. & Gargouri, J. Sperm DNA fragmentation and oxidation are independent of malondialdehyde. *Reproductive Biology and Endocrinology*, 2011, 9:47-54.
14. Aitken, R. J., Whiting, S., Deluliis, G. N., McClymont, S., Mitchell, L. A. & Baker, M. A. Electrophilic aldehydes generated by sperm metabolism active mitochondrial reactive oxygen species generation and apoptosis by targeting succinate dehydrogenase. *Journal of Biology and Chemistry*, 2012, 287:33048-33060.
15. Trevo. www.trevocorporate.com/healthfocusinggredientglossary. Retrieved 17th November, 2014.

16. Pamplona-Roger, G. D. *Encyclopedia of food and their healing power*. Madrid: Editorial Safeliz, 2005.
17. Ekaluo, U. B., Ikpeme, E. V., Ibaing, Y. B. & Omordia, F. O.Effect of soursop (*Annonamuricata* L.) fruit extract on sperm toxicity induced by caffeine in albino rats. *Journal of Medical Science*, 2013a, 13: 67-71.
18. Ekaluo, U. B., Ikpeme, E. V., Ibiang, Y. B. & Amaechina, O. S. Attenuating role of vitamin C on sperm toxicity induced by monosodium glutamate in albino rats. *Journal of Biological Sciences*, 2013b, 13: 298-301.
19. Ikpeme, E. V., Ekaluo, U. B., Udensi, O. U., Ekerette, E. E. Screening fresh and dried fruits of avocado pear (*Perseaamericana*) for antioxidant activities: An alternative for synthetic antioxidant. *Journal of Life Sciences Research and Discovery*, 2014, 1: 19-25.
20. Ekaluo, U. B., Ikpeme, E. V., Etta, S. E., Erem, F. A. & Daniel, I. O. Protective role of soursop (*Annonamuricata* L.) fruit on testicular toxicity induced by caffeine in albino rats. *Journal of Life Sciences Research and Discovery*, 2014, 1: 26-30.
21. Ekaluo, U. B., Ikpeme, E. V., Udensi, O. U., Ekerette, E. E., Usen, S. O. & Usoroh, S. F. Comparative in vitro assessment of drumstick (*Moringaoleifera*) and neem (*Azadiracta indica*) leaf extracts for antioxidant and free radical scavenging activities. Research Journal of Medicinal Plant, 2015a, 9: 24-33.
22. Ekaluo, U. B., Ikpeme, E. V., Ekerette, E. E. & Chukwu, C. I. *In vitro* antioxidant and free radical activity of some Nigerian medicinal plants: bitter leaf (*Vernoniaamygdalina* L.) and guava (*Psidiumguajava* Del.). *Research Journal of Medicinal Plant*,2015b, 9: 215-226.
23. Fridovich, I. Superoxide dismutase. *Advances in Enzymology*, 1974, 41:35-97.
24. Aebi, H. Catalase in vitro. *Methods in Enzymology*, 1984, 105: 121-126.
25. Flohe, L. & Gunzler, W. A. Assays of glutathione peroxidase. *Methods in Enzymology*, 1984, 105:114-121.
26. Ohkawa, T., Ohishi, N. &Yaki, K. Assay for lipid peroxides in animal tissues by thiobabituric acid reaction. *Analytical Biochemistry*, 1979, 95:391-398.
27. Hatice, P., Demir, F. E., Dermits, C. Y., Hussein, A. & Pasaoghi, O. T. The effects of caffeine on oxidative stress in liver and heart tissues of rats. *Turkish Journal of Medical Science*, 2011, 41 (4): 665-671.
28. Souvik, R., Noorjama, R., Faiqa, A., Satyajit, M. & Santanu, S. Naringenin attenuates testicular damage, germ cell death and oxidative stress in streptozotocin induced diabetic rats. *Journal of Applied Biomedicine*, 2013, 11:195-208.
29. Fujii, J., Luchi, Y., Matsuki, S. & Ishii, T. Cooperative function of antioxidants and redox systems against oxidative stress in male reproductive tissue. *Asian Journal of Andrology*, 2003, 5:231-242.
30. Kermal, D. N., Morshedi, M. & Oehninger, S. Effects of hydrogen peroxide on DNA and plasma membrane integrity of human spermatozoa. *Fertility and Sterility*, 2000, 74:1200-1207.
31. Dianzani, M., Muzio, G., Biocca, M. &Canuto. R. Lipid peroxidation in fatty liver induced by caffeine in rats. *International Journal of Tissue Reactions*, 1991, 13:79-85.
32. Annunziata, L., Domenico, F. & Pietro, T. Glycooxidation in diabetes and related disease. *Clinica Chemica Acta*, 2005, 2:236-250.
33. Akondi, R. B., Kumar, P., Annapurna, A. & Manasa, P. Protective effect Rutin and Naringin on sperm quality in streptozotocin (STZ) induced type 1 diabetic rats. *Iranian Journal of Pharmaceutical Research*, 2011,10:583-596.
34. Wang, M. Y. & Su, C. *Cancer preventive effect of Moringacitrifolia*. The proceedings of the Strang international cancer prevention conference, New York, November, 2000, 10-11.