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RESEARCH ARTICLE

Attenuating role of Trévo dietary supplement on hormonal toxicity induced by caffeine in albino rats

¹*Uno, U. U., ²Ekaluo, U. B., ²Okoi, E. P., ¹Ogbe, H. O. and ²Peter, N.

1.Department of Biology, Cross River State College of Education, Akamkpa, Nigeria.

2.Department of Genetics and Biotechnology, University of Calabar, Calabar, Nigeria.

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*Corresponding Author

Uno, U. U.

Abstract

The attenuating role of Trévo dietary supplement (TDS), a multi-herbal health drink on caffeine induced hormonal toxicity in albino rats was studied. 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 experimental animals were treated with combinations of caffeine and TDS orally. The treatment lasted for 65 days. Results indicated statistically significant ($P < 0.05$) reduction in the serum levels of testosterone, follicle stimulating hormone (FSH), luteinizing hormone/interstitial cell stimulating hormone (LH/ICSH) and estradiol in caffeine treated rats. However, TDS effectively attenuated the caffeine induced toxicities on the hormonal milieu in a dose-dependent manner. These results shows that TDS is effective in attenuating caffeine induced hormonal toxicity in albino rat models.

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INTRODUCTION

Caffeine is a natural alkaloid and is one of the world's most widely consumed psychoactive substances present in several foods, drugs and beverage products such as energy drinks, coffee and tea (Best, 1999; Fredholm *et al.*, 1999; Smith, 2002). Unlike most other psychoactive substances, it is legal and unregulated in most part of the world (Ekaluo *et al.*, 2005; Craig, 2008; Ekaluo *et al.*, 2009) with an estimated 80% of the world's population consuming a caffeine-containing substance daily (Best, 1999; Craig, 2008).

Caffeine and other methylxanthines are used in clinical medicines as diuretics, analgesics, muscle relaxants and can aid in the treatment of brain disorders such as headaches and Parkinson's diseases (Kolayli *et al.*, 2014). In humans, low and average doses of caffeine produce increase alertness and positive effects on the myocardium, while high doses causes caffeine dependency with a wide range of unpleasant physical and mental conditions such as nervousness, irritability, restlessness, insomnia, headache and heart palpitations (Lunch *et al.*, 2007). Caffeine has been reported to cause inhibition of Leydig cell differentiation and significant decrease in plasma follicle stimulating hormone and luteinizing hormone (Ezzat and elGohary, 1994; Parazzini *et al.*, 1993).

Consumption of caffeine has also been linked with delayed conception (Bolumaret *et al.*, 1997), reproductive and developmental toxicities (Ekaluo *et al.*, 2013a; b; 2014) and increase in the frequency of sperm abnormalities (Robbins *et al.*, 1997; Ekaluo *et al.*, 2005; 2009).

Trévo dietary supplement (TDS) is a multi-herbal drink containing 174 ingredients from different part of the world that works synergistically together. It contains essential vitamins and minerals as well as vital trace minerals, amino acids, essential fatty acids, antioxidants, digestive enzymes and co-enzymes as shown in Table 1. The sources of these nutrients include phytonutrients from familiar garden fruits and vegetables, herbs, and coral calcium complex. The supplement is said to be vital in promoting good health, increasing energy, enhancing mental focus, weight management, boosting immunity, maintaining effective cardiovascular system etc. (Trevo, 2014). However, there is dearth of published literature on the uses and health benefits associated with the consumption of TDS.

In view of the increasing intake of caffeine and its abuse which is a reoccurring habit that may cause toxicities and other harmful effects, this study is designed to ascertain the attenuating role of TDS on caffeine induced hormonal toxicities in male albino rats as mammalian model, because it's myriad 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 Int'l Lab LLC, TX 75244, USA for TRÉVO LLC™, 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 2.

The rats were sacrificed under chloroform anaesthesia 24 hours after the last treatment. Blood samples were obtained through cardiac puncture for hormonal assay.

Hormonal Assay

The blood samples were spun at 2500rpm for 10 minutes using Wisperfuge Model 1384 centrifuge (Tamson, Holland) at 10-25°C. Serum samples were assayed for levels of testosterone follicle stimulating hormone (

FSH), luteinizing hormone/interstitial cell stimulating hormone (LH/ICSH) and estradiol using the microwell enzyme linked immunoassay (ELISA) technique utilizing the competitive binding principle; with analytical grade reagents from Syntrol Bioresearch Inc. USA (Ekaluo *et al.*, 2010).

Statistical analysis

Data from the levels of testosterone, FSH, LH/ICSH and estradiol were subjected to Analysis 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.

RESULT AND DISCUSSION

Testosterone

The serum level of testosterone significantly ($P < 0.05$) reduced in all the caffeine treated animals when compare with the control. The testosterone level in caffeine group was reduced by 2.74% when compared with the control. Though not significantly, the level of testosterone increased in the C+T₁ and C+T₂ groups (2.78 and 3.62ng/ml, respectively). The highest testosterone level was obtained in T₁ group (5.94ng/ml), followed by the control (4.38ng/ml) as shown in Table 3.

Luteinizing hormone

Table 3 revealed that the level of luteinizing hormone (LH) significantly decreased in the caffeine group (6.15mIU/ml) when compared with the control (9.77mIU/ml) indicating a 37.05% reduction. The effect of caffeine was attenuated by TDS from 6.15mIU/ml to 8.18 and 9.24mIU/ml in C+T₁ and C+T₂ groups, respectively in a dose-dependent manner.

Follicle stimulating hormone

Follicle stimulating hormone (FSH) level significantly reduced in all caffeine treated groups when compared with the control (Table 3). There was a 67.78% reduction in the caffeine group (0.29mIU/ml) when compared with the control (0.90mIU/ml). C+T₁ and C+T₂ groups had 0.59 and 0.70mIU/ml, respectively indicating a dose-dependent attenuating effect of TDS. The highest FSH level was obtained in the T₁ group (1.60mIU/ml).

Estradiol

Results showed that there was a significant decrease in the level of estradiol in the caffeine treated groups when compared with the control as shown in Table 3. The estradiol level was reduced by 44.70% in the caffeine

group (1.88pg/ml) when compared with the control (3.40pg/ml). However, TDS significantly attenuated the effect of caffeine on the level of estradiol in a dose-dependent manner (2.65 and 2.88pg/ml in groups C+T₁ and C+T₂, respectively). The highest estradiol level was obtained in the T₁ group (5.76pg/ml).

Discussion

In the present study, results obtained revealed that caffeine caused a significant ($P < 0.05$) reduction in the serum levels of testosterone, LH/ICSH, FSH and estradiol which agrees with the findings of Mehran *et al.* (2012), Anup *et al.* (2007), Choi *et al.* (2011), Pollard (1990) and Karen *et al.* (2012) who reported an inverse relationship between caffeine intake and the level of sex hormones. The significant reduction in the level of the hormones might be due to degenerations and atrophy in the seminiferous tubules lined by the Sertoli and Leydig cells which plays a vital role in the biosynthesis of reproductive hormones (Souvix *et al.*, 2013; Mehran, *et al.*, 2012, Yoshida, 2002) and/or oxidative damage (Agarwal and Prabakaran, 2005).

FSH and LH work simultaneously with testosterone during spermatogenesis and decrease in the level of these hormones impairs spermatogenesis (O'Shaughnessy *et al.*, 2010; Greenspan and Stawler, 1997; Gelain *et al.*, 2005). Studies have shown that adequate bioavailability of testosterone plays an important role in the structural and functional integrity of the reproductive organs while a decrease is one of the indicators of chemical toxicity to the reproductive system (Mann 1974; Yoshida *et al.*, 2002 and Ono *et al.*, 2004).

However, TDS was found to attenuate the effect of caffeine and also increased the levels of testosterone, FSH, LH/ICSH and estradiol. Epidemiological studies have revealed that consuming fruits and vegetables, as well as the extracts reduces free radical oxidative damage (Wang and Su, 2000). Therefore, the attenuating effect of TDS can be attributed to its rich phytonutrients, vitamins and antioxidants content (Pamplona-Roger, 2005; Thakkar *et al.*, 2011; Ekaluo *et al.*, 2013a; 2014).

Conclusion

The present study shows that Trévo dietary supplement is effective in attenuating caffeine induced hormonal toxicities in albino rat models, in a dose dependent manner.

Table 1: Major ingredients in Trévo dietary supplement(TDS)

13 Essential vitamins,
14 Minerals,
24 Exotic and garden fruits,
18 Vegetables and sea vegetables,
25 herbs,
5 Green super foods,
58 Plant and sea trace minerals,
Plant-source essential fatty acids,
20 Amino acids,
1,000 mg of Coral calcium,
Graviola,
Co-enzyme Q10, and
Fulvicacid

Adapted from: Trevo, 2014

Table 2: Protocol for daily treatment of experimental animals

Treatment groups	Description of treatment
Control	No caffeine and no (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

Table 3: Effect of Trévo dietary supplement(TDS) on caffeine induced toxicities in albino rats

Parameters	Treatment groups				
	C	C+T ₁	C+T ₂	T ₁	Control
Testosterone (ng/ml)	2.74 ^c ±0.34	2.78 ^c ±0.20	3.62 ^c ±0.24	5.94 ^a ±0.32	4.38 ^b ±0.26
LH (mIU/ml)	6.15 ^b ±0.18	8.18 ^b ±0.24	9.24 ^a ±0.25	10.28 ^a ±1.10	9.77 ^a ±0.91
FSH (mIU/ml)	0.29 ^c ±0.05	0.59 ^b ±0.04	0.70 ^b ±0.01	1.60 ^a ±0.23	0.90 ^b ±0.012
Estradiol (pg/ml)	1.88 ^c ±0.15	2.65 ^c ±0.24	2.88 ^c ±0.04	5.76 ^a ±0.39	3.40 ^b ±0.34

Values across the table with similar superscripts are not significantly different at 5% based on ANOVA.

C = Caffeine at 200mg kg⁻¹ BW; T₁= TDS, 1mL kg⁻¹ BW; T₂ = TDS, 2mL kg⁻¹ BW

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