Asian Journal of Clinical Nutrition

ISSN 1992-1470

science alert

ANSInet
an open access publisher
http://ansinet.com

Asian Journal of Clinical Nutrition 7 (3): 84-89, 2015
ISSN 1992-1470 / DOI: 10.3923/ajcn.2015.84.89

Attenuating Potential of Trévo Dietary Supplement on Caffeine Induced Sperm Toxicities in Albino Rats

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ABSTRACT

The attenuating potential of Trévo Dietary Supplement (TDS), a multi-herbal health drink containing 174 ingredients on caffeine-induced sperm toxicities was studied on albino rats. 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 ($p<0.05$) decreases in weight of testes and epididymis, epididymal sperm viability, motility and count in caffeine treated rats. There was a concomitant increase in sperm head abnormalities in caffeine treated rats. However, TDS effectively attenuated the caffeine induced sperm toxicities in albino rat models in a dose dependent manner.

Key words: Caffeine, trévo, dietary supplement, spermatotoxicity

INTRODUCTION

Caffeine is one of the world's most widely consumed psychoactive substances and is 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, 2009; Craig, 2008) 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*., 2004). 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 (Lanch *et al*., 2007).

Consumption of caffeine has also been linked with delayed conception (Bolumar *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).

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, 2012). However, there is dearth of published literature on the uses and health benefits associated with the consumption of TDS.

Table 1: Major ingredients in Trevo 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
Fulvic acid

Adapted from Trevo (2012)

In view of the increasing intake of caffeine and its abuse which is a reoccurring habit that may cause toxicities and mutations. This study is designed to as certain the attenuating potential of TDS on caffeine induced sperm toxicities in male albino rats as mammalian model, because it's 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 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 176.5 g 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 h after the last treatment. The epididymis and testes were dissected out and weighed using Scout Pro SPU 601 electronic weighing balance. The epididymis were processed for epididymal sperm count, motility, viability and sperm head abnormality.

Semen pH and motility: Immediately after dissection, a puncture was made in the epididymis with a sterile pin. The semen smeared on the pin was rubbed on a pH paper of range 4.0-10.0. The colour change corresponding to the pH of the semen was read from the paper. Two drops of sperm suspension were put on a microscope slide and cover slip was placed on it. The number of

Table 2: Protocol for daily treatment of experimental animals

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

progressively motile cell was recorded and divided by the total number of spermatozoa counted under ×40 lenses and expressed in percentage.

Sperm viability: The sperm viability test-was determined using Eosin-Nigrosin staining technique (Bjorndahl *et al*., 2003). A portion of the sperm suspension was mixed with equal volume of Eosin-Nigrosin, stain and air-dried smears were prepared on glass slide for each sample. The slides were examined for percentage viability. Viable sperm cells appeared whitish, while dead sperm cells took up stain and appeared pinkish. The percentage viability was calculated based on the number of viable sperm cells out of the total number of cells observed.

Sperm count: The epididymal sperm samples were obtained by macerating known weights of caudal epididymis in physiological saline in the ratio of 1:10 weight by volume. The epididymis was pipetted to release the sperm cells and filtered using an 80 μm stainless mesh. Epididymal sperm count was obtained by cytometry using the improved Neubauer cytometer and was expressed in million/mL1 of the sperm suspension (Ekaluo *et al*., 2008).

Sperm head abnormality test: A portion of the sperm suspension was mixed with 1% eosin Y solution (10:1) for 30 minutes and air-dried smears prepared on glass slides for the sperm head abnormality test. The slides were examined for percentage sperm head abnormalities in every 200 spermatozoa observed on each slide for each sample. The percentage of sperm head abnormality was calculated according to Ekaluo *et al*. (2009).

Statistical analysis: Data obtained from epididymal semen pH, motility, viability, count, sperm head abnormalities and weight of testes and epididymis 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.

RESULTS

Weight of testes and epididymis: The weight of testes reduced significantly ($p>0.05$) in group of rats treated with caffeine alone (1.07 g) as shown in Table 3, when compared with other treatment groups. The weight of testes in caffeine group was reduced by 11.57% when compared with the control group. Similarly, the weight of epididymis significantly reduced in caffeine treated animals (C, $C+T_1$ and $C+T_2$) when compared to the control (0.05 g). The weight of epididymis in caffeine group was reduced by 28.00% when compared with the control group. The effect of caffeine on the testes and epididymis was attenuated by TDS from 1.07-1.25 g and 0.36-0.42 g, respectively for testes and epididymis.

Semen pH and sperm viability: There was no significant ($p>0.05$) effect of caffeine and TDS on the semen pH. The sperm viability was significantly ($p<0.05$) reduced in rats treated with caffeine. The sperm viability in caffeine group was reduced by 37.56% when compared with the control

Table 3: Effect of Trevo dietary supplement on caffeine induced toxicities in albino rats

	Treatment group				
Parameters	C	C+T_1	C+T_2	T_1	Control
Weight of testes (g)	1.07±0.02^b	1.21±0.02^a	1.25±0.01^a	1.24±0.02^a	1.21±0.05^a
Weight of epididymis (g)	0.36±0.01^c	0.42±0.02^b	0.42±0.01^b	0.51±0.01^a	0.50±0.01^a
Semen Ph	7.07±0.03^a	7.09±0.03^a	7.05±0.02^a	7.01±0.03^a	7.21±0.00^a
Sperm motility (%)	31.03±1.58^c	67.98±0.95^b	62.51±1.81^b	66.14±3.43^b	75.46±1.42^a
Sperm count ($\times10^6$ mL^{-1})	5.06±0.09^d	6.86±0.13^c	7.29±0.14^b	8.31±0.16^a	8.18±0.23^a
Sperm viability (%)	51.38±1.02^d	69.49±1.19^c	72.33±1.11^b	84.21±1.82^a	82.59±1.05^a
Sperm head abnormalities (%)	18.07±0.69^a	10.27±0.60^b	9.57±0.21^b	6.70±0.18^c	7.54±0.29^c

Values across the table with similar superscripts are not significantly different at 5% based on ANOVA, C: Caffeine at 200 mg kg^{-1} b.wt., T_1: TDS, 1 mL kg^{-1} b.wt., T_2: TDS, 2 mL kg^{-1} b.wt.

group. However, TDS significantly attenuated the effect of caffeine in a dose-dependent manner (69.49 and 72.33% for C+T_1 and C+T_2 respectively). The highest percentage of viability was obtained in groups of animals treated with TDS singly and the control (Table 3).

Sperm motility: The sperm motility decreased significantly in the caffeine treated animals when compared with the control (Table 3). The sperm motility in caffeine group was decreased by 58.88% when compared with the control group. The effect of caffeine was attenuated by the different concentrations of TDS from 31.03-67.98 and 62.51%, respectively for C+T_1 and C+T_2.

Sperm count: The sperm count was significantly ($p<0.05$) reduced in caffeine treated groups (caffeine, C+T_1 and C+T_2) when compared with the control ($8.18\times10^6 mL^{-1}$) and TDS ($8.3\times10^6 mL^{-1}$) groups as shown in Table 3. The sperm count in caffeine group was reduced by 38.14% when compared with the control group. TDS also showed attenuating effect on caffeine induced toxicity of sperm count in a dose-dependent manner (6.86 and 7.29×10^6 mL^{-1} in C+T_1 and C+T_2, respectively).

Sperm head abnormality: Results obtained on the effect of TDS on caffeine-induced sperm head abnormality are presented in Table 3. Animals treated with caffeine alone had the highest percentage of sperm head abnormality (18.07%) when compared to other treatment groups. The sperm head abnormality in caffeine group was increased by 139.66% when compared with the control group. The TDS had an attenuating effect on caffeine induced sperm head abnormality in a dose-dependent manner from 18.07-10.27 and 9.57%, respectively in groups C+T_1 and C+T_2, respectively.

DISCUSSION

Results obtained in the study revealed that caffeine caused a significant reduction in the weight of testes and epididymis, sperm count, viability and motility which agrees with the findings of Wilcox *et al*. (1988), Ekaluo *et al*. (2009) and Ikpeme *et al*. (2012) and also significantly increased the percentage of sperm head abnormalities which is similar to the findings of Ekaluo *et al*. (2009, 2013a, b) and Ikpeme *et al*. (2012).

The reduction in the sperm profile of the caffeine-treated animals could be due to the disruption or distortion in the spermatogenic pathways and processes. This is corroborated by Ezzat and El-Gohary (1994) who concluded that long term intake of caffeine induces suppression of spermatogenesis. Ikpeme *et al*. (2010) also showed that the distortions in fertility of male mammals are directly correlated to the distortions in spermatogenesis. In the same vein, decrease in sperm motility suggests alteration of sperm maturation in the epididymis. Also, the increase in the sperm

head abnormality in caffeine treated-animals is indicative of induced mutation on the sperm cells during spermatogenesis as observed by Ekaluo *et al*. (2009), Glover and Assinder (2006) and Ikpeme *et al*. (2010).

The weight of testes and epididymis significantly reduced in groups of animals treated with caffeine. The reduction is confirmed by the significant decrease in the sperm count of animals treated with caffeine (Table 3). This reduction in the weight of testes and epididymis might also be due to distortion in spermatogenesis as earlier stated and/or testicular degenerations as a result of the treatment (Wilcox *et al*., 1988; Dorostghoal *et al*., 2012).

The TDS was found to attenuate the effect of caffeine and also increased the weight of testes and epididymis, sperm motility, sperm count, sperm viability and significantly ($p<0.05$) reduced sperm head abnormality. Epidemiological studies have revealed that consuming fruits and vegetables as well as their extracts reduced free radical oxidative damage (Wang and Su, 2000). Increased Reactive Oxygen Species (ROS) level has been correlated with decreased sperm count and motility (Armstrong *et al*., 1999). Therefore, the attenuating effect of TDS on caffeine induced sperm toxicities can be attributed to its rich phytonutrients, vitamins and antioxidants content (Pamplona-Roger, 2005; Thakkar *et al*., 2011; Ekaluo *et al*., 2013a, 2014), which confer the protective roles against oxidative stress and induced mutations.

CONCLUSION

The present study shows that TDS is effective in attenuating caffeine induced toxicities on weight of testes and epididymis and sperm quality and quantity in albino rat models in a dose dependent manner.

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