Journal of Advances in Biology & Biotechnology

18(3): 1-7, 2018; Article no.JABB.43249
ISSN: 2394-1081

Ameliorating Potential of Honey on Caffeine Induced Sperm Toxicity in Male Albino Rats

U. U. Uno[1,2*], A. J. Umoyen[1], I. S. Esua[1], F. O. Okey[1] and U. B. Ekaluo[1]

[1]Department of Genetics and Biotechnology, University of Calabar, Calabar, Nigeria.
[2]Department of Biology, Cross River State College of Education, Akamkpa, Nigeria.

Authors' contributions

This work was carried out in collaboration between all authors. Author UUU designed the study, performed the statistical analysis, wrote the protocol and wrote the first draft of the manuscript. Authors AJU and ISE managed the analyses of the study. Authors FOO and UBE managed the literature searches. All authors read and approved the final manuscript.

Article Information

DOI: 10.9734/JABB/2018/43249
Editor(s):
(1) Anil Kumar, Professor & Head, School of Biotechnology, Devi Ahilya University, Madhya Pradesh, India.
(2) Maria Serrano, Department of Applied Biology, EPSO, University Miguel Hernandez, Orihuela, Alicante, Spain.
Reviewers:
(1) Raquel Frenedoso da Silva, Brazilian Nanotechnology National Laboratory, Center for Research in Energy and Materials (CNPEM), Brazil.
(2) Azab Elsayed Azab, Sabratha University, Libya.
(3) Noor Hashida Hashim, University of Malaya, Malaysia.
Complete Peer review History: http://www.sciencedomain.org/review-history/26002

Original Research Article

Received 9th June 2018
Accepted 16th August 2018
Published 25th August 2018

ABSTRACT

This study evaluated the ameliorating effect of honey on caffeine-induced sperm toxicity in male 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 honey orally. Group 1 served as the control and was given only water and feed; Group 2 (honey group) received 10 ml/kgBW of honey; Group 3 (caffeine group) received 200 mg/kgBW of caffeine; Group 4 (C + H_1 group) received 200 mg/kgBW of caffeine and 10 ml/kgBW of honey while Group 5 (C + H_2) received 20 ml/kgBW of honey and 200 mg/kgBW of caffeine. The treatments lasted for a period of 65 days. Results showed statistically significant ($p<0.05$) reduction in weight of testes and epididymis, epididymal sperm viability, motility and count in caffeine treated rats when compared with the control. There was a concomitant increase in sperm head abnormalities in caffeine treated rats. However, honey effectively ameliorated the caffeine induced sperm toxicities in albino rat models in a dose-dependent manner.

**Corresponding author: E-mail: ukamuno@yahoo.com;*

Keywords: Caffeine; honey; ameliorating effect; sperm toxicity.

1. INTRODUCTION

Honey is the natural product of bees (Honey bees) formed from the nectar collected from flowering vegetation [1]. It is an contains ingredients similar to those found in fruits, alkaline in the digestive system [2]. From ancient times, honey has been used as both a natural sweetener and a healing agent. Honey is a high nutrient source containing sugars such as glucose and fructose, as well as minerals like magnesium, potassium, calcium, sodium chloride, sulphur, iron, zinc, phosphates and vitamins B1, B2, C, B6, B5 and B3 [3-4].

Honey is a natural product with very complex chemical composition. The composition of a particular honey sample greatly depends on the composition of nectar, where it originates. It contains more than 180 substances including moisture; sugars such as glucose and fructose; enzymes such as catalase and glutathione reductase; trace essential elements such as iron, copper, zinc and calcium; vitamins such as vitamin A, C and E as well as some flavonoids and phenolic acids [5-9]. It is composed primarily of fructose and glucose but also contains 4-5% of fructo-oligosaccharide which serves as a prebiotic agent [10]. Thus, the composition of honey varies with different floral sources as well as climatic and environmental conditions [9,11].

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 [12-14]. Unlike most other psychoactive substances, it is legal and unregulated in most part of the world [15-17] with an estimated 80% of the world's population consuming a caffeine-containing substance daily [12,17]. 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 a headache, and Parkinson's diseases [18]. In humans, low and average doses of caffeine produce increase alertness and positive effects on the myocardium, while high doses cause caffeine dependency with a wide range of unpleasant physical and mental conditions such as nervousness, irritability, restlessness, insomnia, headache and heart palpitations [19]. Consumption of caffeine has also been linked with delayed conception [20], reproductive and developmental toxicities [21-27] and an increase in the frequency of sperm abnormalities [28].

Therefore, this study was carried out to determine the ameliorating potential of honey on the caffeine-induced sperm toxicity in albino rats as a mammalian model.

2. MATERIALS AND METHODS

2.1 Treatments and Other Chemicals

Caffeine was obtained from Sigma-Aldrich (St. Louis, MO, USA), while the honey used for this research work was obtained from the Cross River State Ministry of Agriculture, Calabar. All other chemicals used in this study were of analytical grade.

2.2 Experimental Animals

Thirty healthy male albino rats of 12 weeks old; with an 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 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 (Approval number: CRS/MH/CGS&E-H/Vol.1/46).

2.3 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 h after the last treatment. The epididymes and testes were dissected out and weighed using Scout Pro SPU 601 electronic weighing balance. The epididymes were processed for epididymal sperm count, motility, viability and sperm head abnormality.

Table 1. Protocol for daily treatment of experimental animals for 65 days

S/N	Groups	Description
1.	Control	No honey and no caffeine
2.	H_1	10 ml/kgBW of honey only via oral gavage
3.	C	200 mg/kgBW of caffeine only via oral gavage
4.	C + H_1	10 ml/kgBW of honey and 200 mg/kgBW of caffeine both orally via oral gavage
5.	C + H_2	20 ml/kgBW of honey and 200 mg/kgBW of caffeine both orally via oral gavage

2.4 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, which was read from the paper. Two drops (0.05ml each; that is 0.1 ml) of sperm suspension were put on a microscope slide and coverslip was placed on it. The number of progressively motile cell (cells that swim in a mostly straight line or very large circles) was recorded and divided by the total number of sperm cells counted under ×40 lenses and expressed in percentage.

2.5 Sperm Viability

The sperm viability test-was determined using Eosin-Nigrosin staining technique [29]. A portion (0.1 ml) of the sperm suspension was mixed with equal volume of Eosin-Nigrosin stain (0.05 ml each) and air-dried smears were prepared on a glass slide for each sample. The slides were examined for a percentage of viability. Viable sperm cells appeared whitish, while dead sperm cells took up the 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.

2.6 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 [30]. The epididymis was pipetted to release the sperm cells and filtered using 80 µm stainless mesh. Epididymal sperm count was obtained by using the improved Neubauer cytometer (Model: BR723014) and was expressed in $x10^6 mL^{-1}$of the sperm suspension [30].

2.7 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 sperm cells observed on each slide for each sample. The percentage of sperm head abnormality was calculated according to Ekaluo et al. [16].

2.8 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 was considered if $p<0.05$ while Least Significant Difference (LSD) test was used to separate the means.

3. RESULTS

3.1 The weight of Testes and Epididymes

The weight of testes decreased significantly ($p<0.05$) in group of rats treated with caffeine alone as shown in Table 2 when compared with another treatment group. Similarly, the weight of epididymes significantly reduced in the caffeine group when compared to the control and other treatment indicating the toxic effect of caffeine. The effect of caffeine on the weight of testes and epididymes was ameliorated by honey increasing the weight of testes from 1.25g in the caffeine group to 1.34 and 1.39 g in C + H_1 and C + H_2 groups, respectively. The weight of epididymes also increased from 0.39g in the caffeine group to 0.43 and 0.43 g in C + H_1 and C + H_2 groups, respectively.

3.2 Semen pH and Sperm Motility

There was no significant difference in the pH of the semen among the different treatment groups. The pH of the semen was between the range of 7.18 to 7.25 (Table 2).

Table 2 also shows a significant ($p<0.05$) reduction in the sperm motility of rats treated with

Table 2. Effect of honey on rats exposed to caffeine

Parameters	Control	Caffeine	Honey	C + H_1	C + H_2
Weight of testes(g)	1.31^{a}±0.01	1.25^{b}±0.03	1.33^{a}±0.01	1.34^{a}±0.02	1.39^{a}±0.04
Weight of epididymes (g)	0.43^{a}±0.02	0.39^{b}± 0.03	0.45^{a}±0.01	0.43^{a}±0.03	0.43^{a}±0.02
Semen pH	7.25^{a}±0.03	7.18^{a}±0.02	7.28^{a}±0.02	7.20^{a}± 0.06	7.20^{a}±0.04
Sperm motility (%)	83.97^{b}±1.14	77.28^{c}±1.58	89.22^{a} ± 0.96	82.92^{b} ± 2.69	85.84^{a} ±1.78
Sperm viability (%)	88.42^{a}±2.51	81.33^{b}±1.72	91.39^{a}±2.73	89.69^{a}±0.65	91.30^{a}±1.01
Sperm count (x$10^{6}$$mL^{-1}$)	6.95^{b} ± 0.22	5.76^{c} ± 0.23	7.58^{a} ± 0.20	6.37^{b}±0.32	6.89^{b}±0 17
Sperm head abnormalities (%)	6.23^{b} ± 0.10	7.56^{c} ± 0.32	5.87^{a} ± 0.16	6.88^{b}±0.33	6.68^{b}±0.40

**Means with different superscript letters along each horizontal array differ significantly (p<0.05)*

caffeine alone when compared to the control. The effect of caffeine was ameliorated by honey from 77.28% to 82.92 and 85.84% for C + H_1 and C + H_2, respectively in a dose-dependent manner.

3.3 Sperm Viability

The sperm viability was significantly ($p<0.05$) reduced in rats treated with caffeine alone when compared with the control and other treatment groups. The effect on sperm viability was ameliorated in a dose-dependent manner. The percentage of viable sperm cell increased from 81.33% in caffeine group to 89.69% in C + H_1 group and 91.30% in C + H_2 group (Table 2).

3.4 Sperm Count

Sperm count was significantly ($p<0.05$) decreased in animals treated with caffeine alone when compared with the control group and other groups. Honey also ameliorated the effect of caffeine in C + H_1 and C + H_2, respectively increasing the sperm count from 5.76x$10^{6}$$mL^{-1}$ to 6.37 and 6.89x $10^{6}$$mL^{-1}$, respectively. The ameliorating effect of honey was dose-dependent.

3.5 Sperm Head Abnormalities

Results obtained also revealed that animals treated with caffeine alone (caffeine group) had the highest percentage of sperm head abnormalities when compared to control and other treatment groups. The effect of caffeine was ameliorated by honey in a dose-dependent manner from 7.56% in caffeine group to 6.88 and 6.68% in C + H_1 and C + H_2, respectively (Table 2).

4. DISCUSSION

Findings of this study revealed that caffeine had a significant ($p<0.05$) effect on the different sperm parameters studied. The weight of testes and epididymes, sperm count, motility and viability significantly ($p<0.05$) reduced in caffeine-treated animals when compared with the control. This agrees with the reports of Uno et al. [31], Ekaluo et al. [16,24-26] who observed spermatotoxic effect of caffeine on sperm quality.

The reduction in the sperm profile observed as a result of caffeine treatment can be attributed to its impact on spermatogenesis in the animals which is corroborated by Ezzat and El-Gohary [32] who observed that long-term intake of caffeine suppresses spermatogenesis. In the same vein, Ikpeme et al. [33] noted that disruptions infertility in male mammals are directly correlated with disruptions in spermatogenesis. This, therefore, suggests that caffeine treatment might have altered spermatogenic pathways and processes with a concomitant reduction in sperm parameters. This could be a result of reduction in the level of the hormones due to degenerations and atrophy in the seminiferous tubules lined by the Sertoli and Leydig cells which play a vital role in the biosynthesis of reproductive hormones [27,34]. The reduction in sperm count supports the decrease in weight of epididymes observed in the group of rats treated with caffeine alone which could also imply disruptions in sperm maturation in the epididymes. Degenerative changes in testicular histological of rats treated with caffeine probably caused a decline in the testosterone levels and consequently distorted spermatogenesis [35-36]. This might be the underlying cause of the significant reduction in

the sperm count, and weight of testes and epididymis observed in caffeine-treated animals.

Results obtained also indicated a significant ($p<0.05$) increased in sperm head abnormalities in caffeine treated animals which is denotative of induced mutation on the sperm cells during the spermatogenic processes in line with the observations of Ekaluo et al. [16], Glover and Assinder [37], Uno et al. [31], and Ikpeme et al. [33]. However, honey significantly ameliorated the effect of caffeine in groups of rats treated with a combination of caffeine and honey (C + H_1 and C + H_2 groups) in a dose-dependent manner. The ameliorating potential of honey could be due to its rich mineral content such as potassium, iron, magnesium, etc. as well as natural antioxidant content especially flavonoid and phenolic acids [3,7,38].

Moreover, the ameliorating effect of honey can also be attributed to the presence of enzymes such as catalase, glutathione and reductase which prevents oxidative stress and testicular degeneration with its resultant effect on spermatogenesis and sperm profile. Caffeine has been reported to cause significant reductions in superoxide dismutase, catalase and glutathione concentrations indicating a decrease in antioxidant defense system, increased free radical activities and consequently resulting in oxidative stress [24,28]. This is in view of the fact that reactive oxygen species (ROS) level and oxidative stress have been correlated to decreased sperm count and motility [39]. Epidemiological studies have revealed that consuming vitamins and mineral containing foods, fruit and products such as honey as well as their extracts reduced free radical oxidative damage [40].

5. CONCLUSION

The findings of the present study show that honey is effective in ameliorating caffeine induced toxicities on weight of testes and epididymis and sperm quality and quantity in albino rat models in a dose-dependent manner.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

1. Mahanem M, Siti-Amrah S, Yatiban MK, Hasnan J. Effect of 'Tualang Honey' on spermatogenesis in rats. Proceedings of 1st International Conference on the medicinal uses of honey (from Hive to Therapy). Kota Bharu Kelantan, Malaysia; 2006.
2. Bradley PR. Raw honey and cinnamon. Bournemouth, British Herbal Medicine Association. 2010;1:145-148.
3. Estevinho L, Pereira A, Moreira L, Dias L, Pereira E. Antioxidant and antimicrobial effects of phenolic compounds extracts of Northeast Portugal honey. Food Chem. Toxicol. 2008;46:3774-3779.
4. Syazana NS, Hashida NH, Majid AM, Sharifah HAD, Kamaruddin MY. Effects of gelam honey on sperm quality and testis of rat. Sains Malaysiana. 2011;40(11):1243-1246.
5. White JW. Composition of honey. In: Honey: A comprehensive survey. E. Crane (ed.), Heinemann, London. 1979;157–192.
6. Michalkiewicz A, Biesaga M, Pyrzynska K. Solidphase extraction procedure for determination of phenolic acids and some flavonols in honey. Journal of Chromatography. 2008;1187:18–24.
7. Yao L, Jiang Y, D'Arcy B, Singanusong R, Datta N, Caffin N, Raymont K. Quantitative high-performance liquid chromatography analyses of flavonoids in Australian Eucalyptus honeys. Journal of Agricultural and Food Chemistry. 2004;52:210–214.
8. Al-Waili NS. Effects of daily consumption of honey solution on hematological indices and blood levels of minerals and enzymes in normal individuals. Journal of Medicinal Food. 2003;6:135–140.
9. Gheldof N, Wang XH, Engeseth NJ. Identification and quantification of antioxidant components of honeys from various floral sources. Journal of Agricultural and Food Chemistry. 2002;50: 5870–5877.
10. Chow J. Probiotics and prebiotic: A brief overview. Journal of Renal Nutrition. 2002; 12:76–86.
11. Perez RA, Sanchez-Brunette C, Calvo RM, Tadeo JL. Analysis of volatiles from Spanish honeys by solid phase microextraction and gas chromatography mass spectrometry. Journal of Agriculture and Food Chemistry. 2002;50(9):2633-2637.
12. Best B. Is caffeine a health hazard? Am. J. Psychiatry. 1999;156:223-228.
13. Fredholm BB, Battig J, Holmen A, Nehlig K, Zvartau EE. Actions of caffeine in the

brain with special reference to factors that contribute to its widespread use. Pharmacol. Rev. 1999;51:83-133.

14. Smith A. Effects of caffeine on human behavior. Food Chem. Toxicol. 2002;40: 1243-1255.
15. Ekaluo UB, Uno UU, Edu NE, Ekpo PB, Etta SE, Odok TN. Attenuating potential of Trevo dietary supplement on caffeine induced sperm toxicity in albino rats. Asian Journal of Clinical Nutrition. 2015;7(3):84-89.
16. Ekaluo UB, Ikpeme EV, Udokpoh EA. Sperm head abnormality and mutagenic effects of aspirin, paracetamol and caffeine containing analgesics in rats. Int. J. Toxicol. 2009;7:1-9.
17. Craig A. Caffeine may trigger most migraines. Back Experts. 2008;1:23-25.
18. Kolayli S, Ocak M, Kucuk M, Abbasoglu R. Does caffeine bind to metal ions? Food Chem. 2004;84:383-388.
19. Lanch I, Oimer A, Srous RD. Caffeinism: History clinical features, diagnosis and treatment. In: Caffeine and activation theory: Effects in health and behaviour. Smith BD, Gupta U, Gupta BS. (Eds.) CRS Press, Boca Raton, FL. 2007;331-344.
20. Bolumar F, Olsen J, Rebagliato M, Bisanti L. Caffeine intake and delayed conception: A European multicenter study on infertility and subfecundity. European study group on infertility subfecundity. Am J Epidemiol. 1997;145:324-334.
21. Ekaluo UB, Ikpeme EV, Ibiang YB, Omordia FO. Effect of soursop (*Annona muricata* L.) fruit extract on sperm toxicity induced by caffeine in albino rats. J Med Sci. 2013a;13:67-71.
22. Ekaluo UB, Ikpeme EV, Ibiang YB, Amaechina OS. Attenuating role of vitamin C on sperm toxicity induced by *Monosodium glutamate* in albino rats. J Biol Sci. 2013b;13:298-301.
23. Ekaluo UB, Ikpeme EV, Etta SE, Erem FA, Daniel IO. Protective role of soursop (*Annona muricata* L.) fruit on testicular toxicity induced by caffeine in albino rats. J Life Sci Res Discovery. 2014; 1:26-30.
24. Ekaluo UB, Uno UU, Edu NE, Ekpo PB, Etta SE. Effect of Trevo dietary supplement on caffeine induced oxidative stress in albino rat models. The Pharmaceutical and Chemical Journal. 2016a;3(2):92–97.
25. Ekaluo UB, Uno UU, Edu NE, Ekpo PB, Etta SE, Volunteer BO. Protective role of onion (*Allium cepa*) on caffeine induced sperm toxicity in albino rats. Journal of Applied Life Science International. 2016b; 4(4):1-7.
26. Ekaluo UB, Ikpeme EV, Uno UU, Umeh SO, Erem FA. Protective effect of aqueous guava leaf extract on caffeine induced sperm toxicity in albino rats. Research Journal of Medicinal Plant. 2016c;10(1): 98-105.
27. Uno UU, Ekaluo UB, Okoi EP, Ogbe HO, Peter N. Attenuating role of Trevo dietary supplement on hormonal toxicity induced by caffeine in albino rats. International Journal of Advance Research. 2015;3(11): 586-590.
28. Uno UU, Ekpo PB, Ogbe HO, Okolo, CM, Ekaluo UB. Effect of soursop (*Annona muricata* L.) leaf extract on oxidative stress caused by caffeine in albino rat model. Asian Journal of Biology. 2016;1(2):1–7.
29. Robbins WA, Meistrich ML, Moore D, Hagemeister FB, Weier HU. Chemotherapy induces transient sex chromosomal and autosomal aneuploidy in human sperm. Nat Genet. 1997;16:74-78.
30. Bjorndahl L, Soderlund I, Kvist U. Evaluation of the one-step eosin-nigrosin staining technique for human sperm vitality assessment. Hum. Reprod. 2003;18:813-816.
31. Uno UU, Ogbe HO, Okolo CM, Ekaluo UB, Akwa BU. Effect of soursop (*Annona muricata* L.) leaf extract on sperm toxicity induced by caffeine in albino rats. The Pharmaceutical and Chemical Journal. 2017;4(1):82–87.
32. Ezzat AR, El-Gohary ZM. Hormonal and histological effects of chronic caffeine administration on the pituitary-gonadal and pituitary-adrenocortical axes in male rabbits. Funct Dev Morphol. 1994;4:45-50.
33. Ikpeme EV, Udensi O, Ekaluo UB, Effieneokwu N. Biological response of male Wister rats to crude extract of *Ficus exasperata* (VAHL). Int J Curr Res. 2010;7: 9-13.
34. 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 Bio-medicine. 2013;11:195-208.
35. Mann T. Secretory function of the prostate, seminal vesicle and other male accessory

organs of reproduction. Journal of Reproduction and Fertility. 1974;37:179-188.
36. Yoshida M, Kitani T, Takenaka A, Kudoh K, Katsuda SI, Taya K. Lack of effects of oxolinic acid on spermatogenesis in young adult and aged Wister rats. Food Chem Toxicol. 2002;40:1815-1825.
37. Glover A, Assinder SJ. Acute exposure of adult male rats to dietary phytoestrogens reduces fecundity and alters epididymal steroid hormone receptor expression. J Endocrinol. 2006;189:565-573.
38. Cook NC, Sammon S. Flavonoids: Chemistry, metabolism, cardioprotective effects and dietary sources. The Journal of Nutritional Biochemistry. 1996;7:66–76.
39. Armstrong JS, Rajasekaran M, Chamulitrat W, Gatti P, Hellstrom WJ, Sikka SC. Characterization of reactive oxygen species induced effects on human spermatozoa movement and energy metabolism. Free Radical Biol Med. 1999; 26:869-880.
40. Wang MY, Su C. Cancer preventive effect of Moringa citrifolia. Proceedings of the strang international cancer prevention conference November, 10-11 2000. Health Communication Inc.; 2000.

Peer-review history:
The peer review history for this paper can be accessed here:
http://www.sciencedomain.org/review-history/26002