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Honey ameliorates imbalance between reactive oxygen species and antioxidant enzymes in the testis of sleep deprived rats

Ayodeji J. Ajibare[1], Oyesanmi A Fabunmi[1], Olabode O. Akintoye[1], Babatunde A. Olofinbiyi[2], Luqman A Olayaki[3]

[1]Department of Physiology, College of Medicine, Ekiti State University, P.M.B. 5355, Ado – Ekiti 36001, Nigeria.

[2]Department of Obstetrics and Gynaecology, College of Medicine, Ekiti State University, P.M.B. 5355, Ado – Ekiti 36001, Nigeria

[3]Department of Physiology, Faculty of Basic Medical Sciences, College of Health Sciences, University of Ilorin, Ilorin, Nigeria.

Corresponding Author's Email : Fabunmioyesanmi@gmail.com; Oyesanmi.fabunmi@eksu.edu.ng

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Abstract

Background: Sleep deprivation is the restriction or at times, lack of sleep in an organism as result of lifestyle or environmental factors. It can affect the male reproductive system especially testicular function due to Inflammatory and oxidative stress mediators playing important roles.This study evaluated the ameliorative effect of honey on inflammatory and oxidative stress mediators in sleep deprived adult male wistar rats.

Methods and Material: Twenty-five rats were divided into five groups (n=5) and designated as follows: Group 1: Control, Group 2: sleep-deprived (SD), Group 3: sleep deprived and sleep recovery (SD+SR), Group 4: sleep-deprived honey treated (SD+HONEY), Group 5: sleep-deprived and recovery HONEY treated (SD+SR+HONEY). Rats were sleep deprived for five days, Honey 1ml/kg was given orally for honey treated groups while recovery groups were allowed to recover for five days. At the end of the experiments, cortisol, testosterone, interleukin 6 (IL-6), and c reactive protein (CRP) were analyzed while testicular malondialdehyde (MDA), catalase (CAT), and glutathione (GSH) were also evaluated. Data was expressed as means ± standard error of the mean (SEM). Statistical group analysis was performed with Graph pad (Prism 7) statistical software and statistically significant differences were accepted at $p < 0.05$.

Results: Rats in the SD group had significantly increased levels of MDA and cortisol, IL-6, and CRP levels ($p<0.05$), while showing significantly reduced levels of testosterone, GSH, and CAT ($p<0.05$). Treatment with honey reversed the changes in the MDA, GSH, CAT, cortisol, testosterone, IL 6, and CRP levels ($p<0.05$), while sleep recovery was also helpful in reversing the changes.

Conclusions: Honey administration ameliorated the high levels of testicular inflammatory and oxidative stress markers caused by sleep deprivation.

Keywords: Honey; Inflammation; Oxidative stress; Sleep deprivation; Sleep recovery

INTRODUCTION

Sleep deprivation (SD) can be defined as the restriction or at times, almost -total removal of sleep in an organism. It is reported that about 33% of adults in the United States are sleep deprived and this is associated with numerous health challenges. Furthermore, SD causes deregulation of the hypothalamic-pituitary-adrenal (HPA) axis, hypogonadism, and a resultant deleterious effect on testicular function possibly through oxidative stress and inflammation [1,2]. Sleep deprivation modulates the sympathetic effector pathway to raise inflammatory markers which subsequently impairs testicular functions. On the other hand, oxidative stress causes DNA damage and lipid peroxidation thereby damaging the

testis, also the damage caused by reactive oxygen species is more pronounced in the testis since it is prone to lipid peroxidation due to the abundance of polyunsaturated fats in its membrane [3,5]. Sleep deprivation has become almost inevitable in our modern world, therefore substances that would protect against its deleterious effect on the male gonad would be beneficial and demands investigation.

Great interest has been shown towards natural products with potential polymodal protective effects and in recent times, honey has drawn much attention because irrespective of its various floral origin, it still has a considerable level of biological benefits. Honey was reported to protect the testicular tissue against cigarette smoke, raised testosterone level, reduced free radical generation induced by substances harmful to the testis. It also proved effective in ameliorating the systemic inflammation induced by lipopolysaccharide and carrageenan-induced paw inflammation. These actions were linked to the activity of its potent biomolecules and NF-Kb, Nrf2, and mitogen-activated protein kinases modulation [6–8]. To the best of our knowledge, no study has been done to investigate the effect of honey administration on sleep-deprivation induced testicular oxidative stress and inflammation. We aimed to test the hypothesis that honey could mitigate the hypogonadism caused by sleep deprivation in male rats. To achieve this, we examined the effect of honey administration on selected hormones, oxidative stress, and inflammatory biomarkers.

MATERIALS AND METHODS

ANIMALS

Twenty-five 6 weeks old Wistar rats within the ages of five to six weeks and weighing between 180- 200g were purchased from Ekiti State University, Ado –Ekiti, Ekiti-State, Nigeria, and used for the study. They were housed and maintained in standard conditions of light, feeding, and temperature in the Animal House of the College of Medicine, Ekiti State University, Ado –Ekiti, Ekiti-State, Nigeria. The experimental protocol was approved by the Ekiti State University, with protocol number EKSU/A67/2019/02/008. The experimental protocol lasted seventeen days. Rats were acclimatized for seven days. After acclimatization, they were randomly assigned to one of the following experimental groups (n = 5 per group) and treated accordingly. Rats in Group I (Control) had distilled water (10ml/kg, orally) daily. Rats in Group II had distilled water (10ml/kg, orally) daily; designated as Sleep Deprived group (SD). Those in Group III received distilled water (10ml/kg, orally) daily; designated as Sleep Deprived and Recovery group (SD + SR). Those in Group IV received Honey (1g/kg body weight, orally) daily; designated as Sleep Deprived with honey administration (SD + H). Group V received honey (1g/kg body weight, orally) daily; designated as Sleep Deprived and Sleep Recovery with honey administration (SD + SR + H). Rats were exposed to a 12+/-1 hour light-dark cycle to maintain circadian rhythm throughout the experimental period and had unrestricted access to standard rat chow by placing chow pellets and water bottles on a grid located on top of the tank. Daily change of water in the tank was ensured.

HONEY

Nigerian Honey was purchased from the Department of Agriculture at Ekiti State University. Ado Ekiti Ekiti State Nigeria. The honey was diluted with distilled water (1: 1). The dose (1.0 g/kg) was chosen [9]

SLEEP DEPRIVATION MODEL

After acclimatization, the animals were subjected to sleep deprivation using the Modified Multiple Platform (MMP) methods described by [10]. Water tanks with circular platforms inside were used for MMP. The tank is filled with water to about 1 cm from the platforms. The rats were allowed to move around freely inside the tank by jumping from one platform to another. Each tank had platforms upon with rats were placed. Rats were sleep-deprived for 20hrs (11:00 am-7:00 am next morning) for 5 days by making them stand on top of platforms

with water up to 1/3rd of the level of the platforms. Once the rats fall asleep and their tails or head touches the water due to muscle atonia, they wake up. They however had a 4hr (7:00 am-11:00 am) rest each day. The control group was allowed to sleep in their cages. The experiment lasted 5 days for sleep-deprived groups and 10 days for sleep-deprived and recovery model where the animals were given a sleep recovery period of 5 days after sleep deprivation.

DETERMINATION OF BIOCHEMICAL PARAMETERS

Rats were sacrificed in stages: Sleep-deprived groups at the end of five days of sleep deprivation and sleep recovery groups at the end of five days of sleep recovery. Animals were anesthetized using 25% (w/v) urethane and 1% (w/v) alpha chloralose 5ml/kg (BDH Chemicals Ltd., Poole, England) intraperitoneally then humanely sacrificed. Samples were collected via cardiac puncture with 5mls syringes after the opening of the upper abdominal region. Blood samples were collected into plain bottles and centrifuged at 3000r/p for 10 minutes. The serum samples were micro-pipetted into plain bottles and were immediately stored at -4^0c. Afterwards, the serum was used then used to estimate testosterone, cortisol, interleukin 6, and C reactive protein using ELISA kits according to the manufacturer's instructions.

HOMOGENIZATION OF TESTICULAR TISSUE SAMPLES

To estimate Malondialdehyde, Glutathione, and Catalase concentration, the testicles were quickly excised, and thereafter, they were washed in cooled 0.15M NaCl and were homogenized in 2ml of ice-cold potassium phosphate buffer (0.1M, pH: 7.4) using an improvised homogenizer. Samples were centrifuged at 5000r/m for 15 minutes to obtain a supernatant. The supernatant obtained was micro pipetted into 3 different plain bottles. Testicular malondialdehyde, glutathione, and catalase were estimated using ELISA kits according to the manufacturer's instructions.

STATISTICAL ANALYSIS

Data are expressed as means ± standard error of the mean (SEM). Statistical group analysis was performed with Graph pad (Prism 7) statistical software. Test of variance was done using One-way ANOVA, Bonferroni's multiple comparisons test, and statistically significant differences were accepted at $p < 0.05$.

RESULTS

Table 1 shows a significant rise ($P<0.05$) cortisol of SD when compared with CONTROL. There was however a significant reduction ($P<0.05$) in the cortisol level of SD+SR, SD+HONEY, and SD+SR+HONEY when compared to the Group SD. Also, there was a significant reduction ($P<0.05$) in the level of testosterone in SD, when compared to the CONTROL while SD+SR, SD+HONEY, SD+SR+HONEY all showed a significant increase in testosterone level ($P<0.05$) compared to SD.

Effect of honey on the hormonal profile of sleep-deprived male rats

	CONTROL	SD	SD+SR	SD+HONEY	SD+SR+HONEY
CORTISOL(ng/ml)	67.42±4.001	138.8±4.765[a]	100±5.895[b]	106±5.67[b]	90.80±4.398[b]
TESTOSTERONE (ng/ml)	1.812±0.074	1.313±0.057[a]	1.754±0.059[b]	1.702±0.O88[b]	1.962±0.052[b]

Data expressed are means ± SEM, n = 25. Data was analyzed using two ways ANOVA, Bonferroni's multiple comparisons test and p-value equals < 0.05. $_{a,b,c}$ $p < 0.05$ vs control, SD group and SD+SR group. Key: Sleep deprivation (SD); Sleep deprivation and Sleep recovery (SD+SR) Key: Sleep deprivation (SD); Sleep deprivation and Sleep recovery (SD+SR)

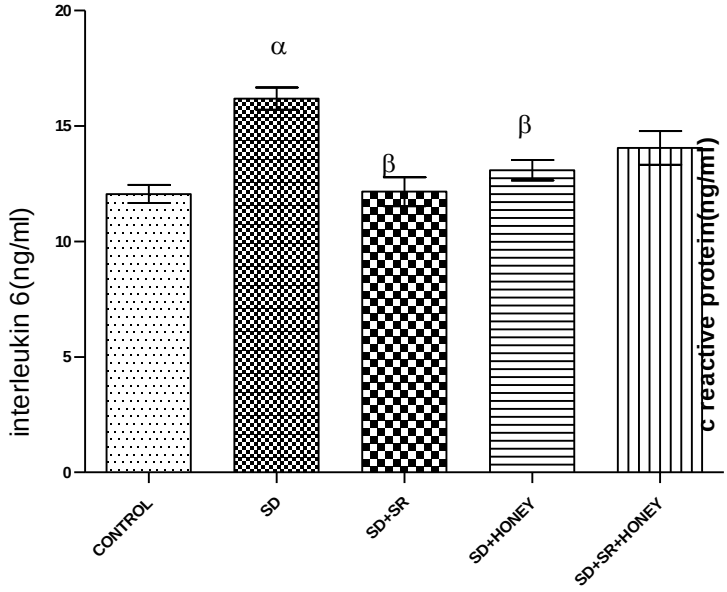
Table 2 shows a significant increase in both MDA and GSH levels (P<0.05) in the SD group when compared to the CONTROL. There is a significant reduction in MDA levels (P<0.05) in SD+SR, SD+HONEY, SD+SR+HONEY groups, compared to SD. However, for CAT, this study revealed a significant reduction (P<0.05) in SD and SD+SR groups in comparison to the CONTROL, a significant increase (P<0.05) in SD+HONEY, SD+SR+HONEY groups compared to the SD group and it also revealed a significant rise (P<0.05 in SD+SR+HONEY group compared to SD+SR group.

Effect of honey on testicular oxidative stress biomarkers of sleep-deprived rats

	CONTROL	SD	SD+SR	SD+HONEY	SD+SR+HONEY
MDA(µM)	27.26±0.987	44.66±1.515[a]	27.56±0.968[b]	32.50±2.628[b]	26.84±1.065[b]
GSH (mM)	1.563±0.043	1.197±0.039[a]	1.420±0.063[b]	1.483±0.047[b]	1.496±0.041[b]
CAT(µmol/min/ml)	46.21±2.797	30.76±1.691[a]	34.23±2.769[a]	44.61±1.482[b]	38.02±1.903[bc]

Data expressed are means ± SEM, n = 25. Data was analyzed using two ways ANOVA, Bonferroni's multiple comparisons test and p-value equals < 0.05. $_{a,b,c}$ $p < 0.05$ vs control, SD group and SD+SR group. Key: Sleep deprivation (SD); Sleep deprivation and Sleep recovery (SD+SR) Key: Sleep deprivation (SD); Sleep deprivation and Sleep recovery (SD+SR)

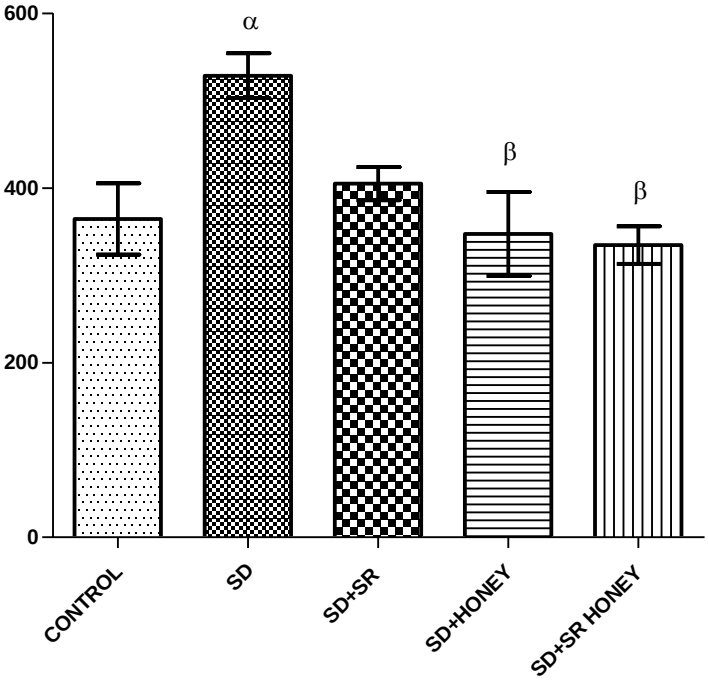
Figure 1 showed a significant rise in IL-6 (P<0.05) in the SD group compared to the CONTROL. There was a significant reduction in IL-6 of SD+SR, SD+HONEY groups compared to SD.



Effects of honey on interleukin 6 levels in normal and sleep-deprived/ recovery male Wistar rats.

Test of variance was done using one-way ANOVA, Bonferroni's multiple comparisons test, and p-value equal < 0.05. $_{a,b,c}$ p < 0.05 vs control, SD group and SD+SR group. Key: Sleep deprivation (SD); Sleep deprivation and Sleep recovery (SD+SR) Key: Sleep deprivation (SD); Sleep deprivation and Sleep recovery (SD+SR)

Figure 2 showed a significant rise in the level of CRP (P<0.05) in the SD group compared to the CONTROL. There was a significant reduction in the CRP levels of HONEY, SD+SR+HONEY groups compared to SD.



Effects of honey on C reactive protein level in normal and sleep-deprived/ recovery male Wistar rats.

Test of variance was done using ANOVA, Bonferroni's multiple comparisons test and p-value equals < 0.05. $_{a,b,c}$ p < 0.05 vs control, SD group and SD+SR group. Key: Sleep deprivation (SD); Sleep deprivation and Sleep recovery (SD+SR) Key: Sleep deprivation (SD); Sleep deprivation and Sleep recovery (SD+SR)

DISCUSSION

The findings from this study suggest that honey could be protective against testicular oxidative stress, and inflammatory effects in the male Wistar rats exposed to sleep deprivation.

Our result showed an inversely proportional relationship between the serum cortisol level and serum testosterone level in the sleep-deprived rats. This is probably because, in chronic stress situations such as sleep deprivation, there is a sustained elevated cortisol production. This sustained rise in cortisol

level causes a state of programmed cell death in the testosterone-producing Leydig cells of the testis thereby resulting in diminished testosterone production [11]. However, in this study, honey suppressed these SD-induced hormonal alterations and significantly reduced serum cortisol while it increased the serum testosterone level in honey-treated animals. This agrees with other animal studies where honey was administered to rats undergoing stressful conditions such as jumping exercises and noise-induced stress [12,13]. It is plausible that honey normalized cortisol level and reduced Leydig cell apoptosis by modulating antiapoptotic patterns in the Leydig cell thereby increasing testosterone level.

Sleep recovery normalized the derangement in cortisol and testosterone level in our study possibly because rats in recovery accommodate the HPA axis derangements that accompany sleep deprivation, therefore, making them have a normally functioning HPA axis during periods of sleep recovery [14] Besides, testosterone is said to rise after about three hours of slow-wave sleep[15,16] Honey significantly improved cortisol and testosterone level during recovery, this agrees with[17] where it facilitated restorative sleep by inducing melatonin release which might modulate cortisol response.

Sleep deprivation could increase oxidative stress by generating Reactive oxygen species (ROS) due to the increased demand for oxygen during the period of wakefulness while exhausting the antioxidant system in the process[18]. ROS can cause cytotoxicity, and one of the ways this is seen is by lipid peroxidation[19]. An increase in MDA level means an increase in the levels of lipid peroxidation and this could prove useful in the assessment of infertility in people with asthenozoospermia [20]

Glutathione (GSH) is a key player in the antioxidant defence system and its major role in removing hydrogen peroxide and organic peroxides from the cell. It is therefore expected that a significant reduction in GSH in a tissue indicates an increase in ROS [15]. The relationship between GSH and defective sperm morphology was highlighted by[5].

Catalase (CAT) is an essential part of the cellular defence against ROS. CAT scavenges hydrogen peroxide forming water and oxygen in the process[21]. There seem to be a negative relationship between the level of catalase in the semen and infertility as low levels were associated with infertility [22]. This further strengthens the link between ROS and diminished testicular function. The findings from this study are consistent with[23] which demonstrated that sleep-deprived male rats had higher levels of MDA but low testicular tissue GSH and CAT levels Furthermore,[24] revealed a decrease in hepatic glutathione after 5 days of sleep deprivation which was worsened by prolongation of sleep deprivation.

Although sweetened beverages and sweets have been reported to have negative effects on the testicles probably due to oxidative stress, research evidence supports the use of Honey to protect the male gonad despite being naturally sweet [25,26]. Also, unlike honey, many of these sweets and sweetened beverages contain substances that have been termed to be contaminants and capable of altering the hypothalamic-pituitary-gonadal function[27]. Honey reversed the alterations in oxidative stress markers in this study just as it was found effective in cigarette smoke-induced oxidative stress by increasing the bioavailability of antioxidant enzymes thereby offering a boost to cope with the destructive ability of ROS [28]. Specific mechanisms by which honey achieves its antioxidants actions are not clear however, free radical sequestration, hydrogen donation, and superoxide radical actions are possible mechanisms [29] Furthermore, constituents of honey such as vitamin c and glutathione reductase may play significant roles. Of note also are the roles performed by both flavonoids and phenolic compounds. The latter aids the tissue antioxidant enzymes while flavonoids are said to upregulate expression of γ- glutamylcysteine synthetase- a key in the synthesis of glutathione [30].

The recovery group of sleep-deprived rats with or without honey supplementation demonstrated a restored antioxidant level more so leads to a decrease in free radical production

since sleep deprivation has stopped. However, these findings corrobartaed with previous animal studies [15,31,32] where beneficial effect of honey was demonstrated to improve antioxidant profile.

Sleep deprivation can induce inflammation [5] by activating the p38 MAPK pathway due to the release of epinephrine which binds to β2-ARs, the p38MAPK pathways ultimately enhance nuclear factor kappa B (NF-κB) and cause cytokines and chemokines expression [33]. Furthermore, the testicles do not have a strong inflammatory immune response therefore, sustained inflammation as seen in sleep deprivation might engulf the immune suppressor system leading to an autoimmune state within the testis further disrupting testicular functions [34].

Interestingly, honey attenuated the raised pro-inflammatory markers in this study and the earlier work of [8] where stingless bee honey was reported to have decreased the circulating levels of CRP, IL-1β, IL-6, and MCP-1, and reduced NF-κB and p38 MAPK signalling in different tissues of chronic subclinical inflammation model agrees with these findings, besides [35] also reported that honey significantly reduced pro-inflammatory markers in the brain of rats exposed to chronic stress. Phenolic compounds play a significant role in the anti-inflammatory action of honey[36,37], For example, chrysin and quercetin (examples of phenolic compounds present in honey) suppressed lipopolysaccharide-induced COX-2 in Raw 264.7 cells and reduced human CRP expression in mice respectively[38]. Rats in recovery possibly have a gradual stabilization of the hypothalamic-pituitary-adrenal axis which halted the production of cytokines through the p38 MAPK pathway and this halted cytokine expression through the p38MAPK pathway as observed by [16]. Furthermore, from this study, honey is poised to aid the resolution of inflammation by probably boosting the effect of sleep recovery since rats in recovery without honey supplementation had no significant reduction in the level of CRP although this was not significant in the interleukin 6 levels.

In conclusion, sleep deprivation has a serious negative effect on the testis through various mechanisms such as oxidative stress and inflammation, however according to our study honey mitigated sleep deprivation-induced hypogonadism possibly by restoring hormonal levels, normalizing inflammatory and oxidative stress biomarkers.

ETHICAL APPROVAL

The experimental protocol was approved by the Ekiti State University, with protocol number: EKSU/A67/2019/02/008

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REFERENCES

1. Dai H, Hao J. Sleep Deprivation and Chronic Health Conditions Among Sexual Minority Adults. Behav Sleep Med 2019;17:254–68.

2. Wittert G. The relationship between sleep disorders and testosterone in men. Asian J Androl 2014;16:262.

3. Irwin MR, Olmstead R, Carroll JE. Sleep Disturbance, Sleep Duration, and Inflammation: A Systematic Review and Meta-Analysis of Cohort Studies and Experimental Sleep Deprivation. Biol Psychiatry 2016; 80:40–52.

4. O'Bryan MK, Schlatt S, Gerdprasert O, Phillips DJ, de Kretser DM, Hedger MP. Inducible nitric oxide synthase in the rat testis: evidence for potential roles in both normal function and inflammation-mediated infertility. Biol Reprod 2000;63:1285–93.

5. Rizk NI, Rizk MS, Mohamed AS, Naguib YM. Attenuation of sleep deprivation dependent deterioration in male fertility parameters by vitamin C. Reprod Biol Endocrinol 2020;18:1–13.

6. Banihani SA. Mechanisms of honey on testosterone levels. Heliyon 2019;5:e02029.

7. Owoyele BV, Adenekan OT, Soladoye AO. Effects of honey on inflammation and nitric oxide production in Wistar rats. Zhong Xi Yi Jie He Xue Bao 2011; 9:447–52.

8. Ranneh Y, Akim AM, Ab Hamid H, Khazaai H, Fadel A, Mahmoud AM. Stingless bee honey protects against lipopolysaccharide induced-chronic subclinical systemic inflammation and oxidative stress by modulating Nrf2, NF-κB and p38 MAPK. Nutr Metab 2019;16:15.

9. Erejuwa OO, Sulaiman SA, Ab Wahab MS, Sirajudeen KN, Salleh S, Gurtu S. Honey supplementation in spontaneously hypertensive rats elicits antihypertensive effect via amelioration of renal oxidative stress. Oxid Med Cell Longev 2012;2012.

10. Oh MM, Kim JW, Jin MH, Kim JJ, Moon DG. Influence of paradoxical sleep deprivation and sleep recovery on testosterone level in rats of different ages. Asian J Androl 2012;14:330–4.

11. Gao H-B, Tong M-H, Hu Y-Q, Guo Q-S, Ge R, Hardy MP. Glucocorticoid induces apoptosis in rat Leydig cells. Endocrinology 2002;143:130–8.

12. Mosavat M, Ooi FK, Mohamed M. Stress hormone and reproductive system in response to honey supplementation combined with different jumping exercise intensities in female rats. BioMed Res Int 2014;2014.

13. Rajabzadeh A, Sagha M, Gholami MR, Hemmati R. Honey and Vitamin E Restore the Plasma Level of Gonadal Hormones and Improve the Fertilization Capacity in Noise-Stressed Rats. 2015;2:64–8. Crescent Journal of Medical and Biological Sciences.

14. Nirupama M, Yajurvedi H. Efficacy of Ashwagandha (Withania somnifera L.) root extracts in preventing stress-induced testicular damage in rat. Eur J Biomed Pharm Sci 2015;2:413–24.

15. El-Aziz EAA, Mostafa DG. Impact of sleep deprivation and sleep recovery on reproductive hormones and testicular oxidative stress in adult male rats. AAMJ 2012;10:1.

16. Leenaars CH, Dematteis M, Joosten RN, Eggels L, Sandberg H, Schirris M, et al. A new automated method for rat sleep deprivation with minimal confounding effects on corticosterone and locomotor activity. J Neurosci Methods 2011;196:107–17.

17. Akanmu MA, Olowookere TA, Atunwa SA, Ibrahim BO, Lamidi OF, Adams PA, et al. Neuropharmacological effects of Nigerian honey in mice. Afr J Tradit Complement Altern Med 2011;8.

18. D'Almeida V, Hipólide DC, Azzalis LA, Lobo LL, Junqueira VB, Tufik S. Absence of oxidative stress following paradoxical sleep deprivation in rats. Neurosci Lett 1997;235:25–8.

19. Jana K, Samanta PK. Evaluation of single intratesticular injection of calcium chloride for nonsurgical sterilization in adult albino rats. Contraception 2006;73:289–300.

20. Tavilani H, Doosti M, Saeidi H. Malondialdehyde levels in sperm and seminal plasma of asthenozoospermic and its relationship with semen parameters. Clin Chim Acta 2005;356:199–203.

21. Smith CM, Marks AD, Lieberman MA, Marks DB. Marks' basic medical biochemistry: a clinical approach. Lippincott Williams & Wilkins; 2005.

22. Rubio-Riquelme N, Huerta-Retamal N, Gómez-Torres MJ, Martínez-Espinosa RM. Catalase as a Molecular Target for Male Infertility Diagnosis and Monitoring: An Overview. Antioxidants 2020;9:78.

23. Akindele OO, Kunle-Alabi OT, Adeyemi DH, Oghenetega BO, Raji Y. Effects of vitamin E and melatonin on serum testosterone level in sleep-deprived Wistar rats. Afr J Med Med Sci 2014;43:295–304.

24. Everson CA, Laatsch CD, Hogg N. Antioxidant defence responses to sleep loss and sleep recovery. Am J Physiol-Regul Integr Comp Physiol 2005;288:R374–83.

25. Salas-Huetos A, Bulló M, Salas-Salvadó J. Dietary patterns, foods and nutrients in male fertility parameters and fecundability: a systematic review of observational studies. Hum Reprod Update 2017;23(4):371–89.

26. Waykar BB, Alqadhi YA. Protective Role of Royal Jelly and Honey against Cisplatin Induced Sperm Function Parameters in Male Rats. Indian J Public Health Res Dev 2020;11:153–8.

27. Caporossi L, Alteri A, Campo G, Paci E, Tranfo G, Capanna S, et al. Cross-Sectional Study on Exposure to BPA and Phthalates and Semen Parameters in Men Attending a Fertility Center. Int J Environ Res Public Health 2020;17:489.

28. Mohamed M, Sulaiman SA, Jaafar H, Sirajudeen KNS. Antioxidant protective effect of honey in cigarette smoke-induced testicular damage in rats. Int J Mol Sci 2011;12:5508–21.

29. Ahmed S, Sulaiman SA, Baig AA, Ibrahim M, Liaqat S, Fatima S, et al. Honey as a potential natural antioxidant medicine: an insight into its molecular mechanisms of action. Oxid Med Cell Longev 2018;2018.

30. Moskaug JØ, Carlsen H, Myhrstad MC, Blomhoff R. Polyphenols and glutathione synthesis regulation. Am J Clin Nutr 2005;81:277S-283S.

31. Olayaki LA, Sulaiman SO, Anoba NB. Vitamin C prevents sleep deprivation-induced elevation in cortisol and lipid peroxidation in the rat plasma. Niger J Physiol Sci 2015;30:5–9.

32. Bogdanov S, Jurendic T, Sieber R, Gallmann P. Honey for nutrition and health: a review. J Am Coll Nutr 2008;27:677–89.

33. Kolmus K, Tavernier J, Gerlo S. β2-Adrenergic receptors in immunity and inflammation: stressing NF-κB. Brain Behav Immun 2015;45:297–310.

34. Huo M, Han H, Sun Z, Lu Z, Yao X, Wang S, et al. Role of IL-17 pathways in immune privilege: an RNA deep sequencing analysis of the mice testis exposure to fluoride. Sci Rep 2016;6:1–12.

35. Asari MA, Zulkaflee MH, Sirajudeen KNS, Yusof NAM, Sairazi NSM. Tualang honey and DHA-rich fish oil reduce the production of pro-inflammatory cytokines in the rat brain following exposure to chronic stress. J Taibah Univ Med Sci 2019;14:317–23.

36. Ahmad A, Khan RA, Mesaik MA. Anti-inflammatory effect of natural honey on bovine thrombin-induced oxidative burst in phagocytes. *Phytother Res Int J Devoted Pharmacol Toxicol Eval Nat Prod Deriv* 2009;23:801–8.

37. Bean A. Investigating the anti-inflammatory activity of honey. 2012;

38. Huong N, Panyoyai N, Kasapis S, Pang E, Mantri N. Honey and its role in relieving multiple facets of atherosclerosis. *Nutrients* 2019;111–22.