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Supplementation of bee bread decreases the abnormal sperm without affecting the sperm count of Sprague-Dawley rats

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Traditionally, people consumed bee bread for male fertility enrichment and used mainly in folk medicine. The benefit of bee bread and its underlying potential mechanism for protecting or improving sperm parameters have not been well studied. Hence, this study aimed to determine the effect of bee bread supplementation on male reproductive organs' weight, sperm count, and sperm morphology in adult Sprague-Dawley rats. 24 male rats were randomly divided into four groups, which consist of control and treated groups comprised of 6 male rats in each group. The control rats were administered force-feeding by using a syringe, and oral gavages needle with 1ml of distilled water, meanwhile for the treated groups were administered at 1g/kg of bee bread, 2g/kg of bee bread and 3g/kg of bee bread diluted in distilled water for 28 days of treatment. From the result, there are no significant differences observed between the mean weight of testis, seminal vesicle, epididymis, and prostate gland of the control group and the treatment groups. Apart from sperm morphology, no significant differences were apparent in sperm count when compared between all groups. In conclusion, this study showed that bee bread might escalate the sperm parameter, which is sperm morphology. This study suggests the presence of an androgenic effect in bee bread.

Keywords: Androgenic effect, bee bread, male fertility, male reproductive system, sperm count, rat

INTRODUCTION

Bee bread is a product produced from bee pollen that acts as raw material. Bee bread originated from bees collecting pollen to make a Lacto-fermented, enzymatically-activated food. Besides, bee bread ingredient is an “alchemical” bee creation and one of the bee products. Bees produced bee bread by combining honey and bee saliva to pollen and kept the mixture in the cell of the honeycomb (Vialli, 2014; Barene et al. 2015). There is also a presence of tocopherol, thiamine, niacin, polyphenols, folic acid, phytosterols, carotenoid pigments, enzymes, and coenzymes in bee bread (Bakour et al. 2017). Bee bread also has protein, amino acids, fatty acids, carbohydrates, mineral salts, polyphenols, and flavonoids, but all of the nutrients are depending on the natural source of bee pollen (Urcan et al. 2017).

Bee products, which are honey and bee pollen, have been commonly used in diet complements as well as in the enhancement of male fertility. Variety of nutrients such as amino acids, B-complex vitamins, antioxidants such as flavonoid and others in honey and pollen are critical to maintaining proper hormone metabolism, sperm formation and improvement of the sperm parameter (Rasekh et al. 2015; Ceksterytë et al. 2006; Baltrušaitytė et al., 2007, Said et al. 2004; Sharma & Agarwal, 1996; Wong et al. 2002). However, to date, there is a lack of study using bee bread alone to show the effectiveness of bee bread. For example, consumption of pollen could be caused a beneficial effect on sperm production, improved DNA quality with a concomitant increase in the weight of testis and epididymis, sperm count, motility and morphology (Selmanoglu et al. 2009; Mehraban et al. 2014; Bahmanpour et al. 2006; Rasekh et al. 2015). It is proved that the consumption of honey significantly increases the relative weight of the epididymis, epididymal sperm count, and the number of sperm with normal morphology (Abdul-Ghani et al., 2008; Syazana et al. 2011; Igbokwe et al. 2013).

This research is done to measure the effectiveness of bee bread in protecting or improving sperm parameters and fertility as the role of bee bread, and its underlying potential mechanism in protecting or enhancing sperm parameters has not been reported. Bee bread probably has an integral part in protecting and improving the male reproductive system from infertility. This present study aims to determine the effect of bee bread to the male reproductive organs weight (testis, epididymis, prostate gland, and seminal vesicle) and sperm parameters (sperm morphology and sperm count) in adult male rats.

MATERIALS AND METHODS

Animal treatment

In this study, 24 adult male Sprague-Dawley rats aged 8-10 weeks about 200 - 340 in weight were used as the animal model. Animals were maintained as per standard guidelines and protocols. All animals were kept in clean cages and maintained in a controlled and well-ventilated animal room at 25 ± 2 ˚C with 12-h light/12-h dark cycles. Food pellets and water were supplied to all animals ad libitum. The animals were randomly divided into 4 groups (n=6/group), control group, 1g/kg body weight of bee bread group, 2g/kg body weight of bee bread group, 3g/kg body weight of bee bread group.

Before the treatment started, each rat was weighed. In this study, a dose of bee bread was calculated according to the dose treatment which T1 (1 g/kg of bee bread), T2 (2g/kg of bee bread), and T3 (3 g/kg of bee bread) groups by oral gavages once daily for 28 days (OECD, 2008). The consumption of bee bread considered in this study was according to the previous research (Zakaria & Haron, 2018). The dose of bee bread was calculated based on human consumption, which is one tablespoon for every day or approximately 30g/60kg body weight (Kieliszek et al., 2018). Meanwhile, distilled water was given to rats in the control group. The condition and body weights of the animals were recorded daily. After 28 days of treatment, all animals were sacrificed. Testis, epididymis, seminal vesicle and prostate gland were dissected out, trimmed off the attached tissue, and weighed as shown in Figure 1. The results of the weight organs were recorded.

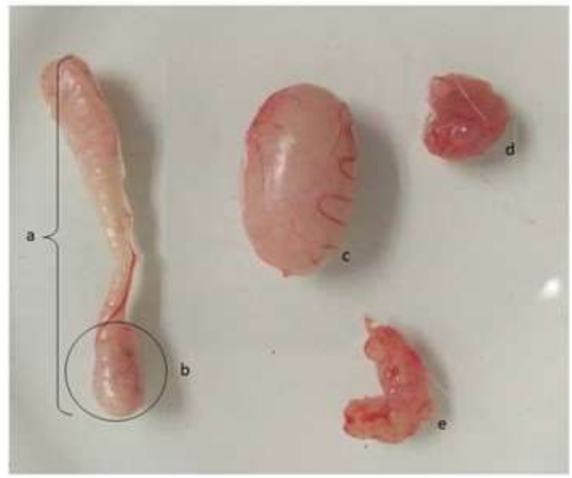

Figure 1. Reproductive organs were taken and weighted. a) epididymis; b) cauda epididymis; c) testis; d) prostate gland; e) seminal vesicle

Sperm analysis

For sperm count and sperm morphology evaluations, cauda epididymis was dissected and minced with scissors in 2 ml normal saline. Then, the mixture was filtered with 80 mm nylon mesh and mixed with 2 drops of Eosin Y. The mixture was left for 30 minutes. This mixture was used for sperm count and the percentage of abnormal sperm morphology.

After 30 minutes, the epididymal content was diluted 20 times with normal saline by using a ratio of 1:20. The dilution was dropped into Neubauer's chamber after placed the cover slide above the chamber. The haemocytometer was placed under the light microscope and viewed under ×400 magnification, and the sperm suspension was counted. The sperm were numbered from 5 chosen small squares out of 25 large squares. The sperm was calculated by counting sperm's head in the square an on the line of the square. To get the concentration of the original sperm sample, then it should be multiplied by the dilution factor. The count was repeated 5 times for each rat to minimise the error.

Sperm morphology was determined by observing the thin smear of the epididymal sperm sample under a light microscope. Firstly, 1 drop of the mixture of minced cauda epididymis was dropped on the slide. The mixture was smeared across the slide by using another slide. After that, the slide was kept air dry for 1 day. The number of abnormal sperm morphology was counted randomly within 200 sperm, which was observed from the smear (Haron et al., 2010).

Data Analysis

Computerised statistical analysis was performed using IBM SPSS Statistics for Windows, Version 19. Experimental data were statistically analysed using One Way ANOVA and expressed as mean ± SEM. Statistical significance was accepted at $p < 0.05$.

RESULTS

There is no significant difference in the mean weight of male reproductive organs within all groups (Table 1). Even though testis weight was lower in the treated group compared to the control group, but the difference was not statistically significant.

In the present study, sperm counts were higher in rats treated with bee bread compared to the control group (Table 2). However, the difference was not statistically significant.

The percentage of abnormal sperms was significantly lower in T1 (1 g/kg of bee bread), T2 (2g/kg of bee bread), and T3 (3 g/kg of bee bread) groups compared to the control group. For each subject, 200 sperm were counted, and these sperm were assessed morphologically. The normal sperm should have a hook-shaped head with a long tail (Figure 2). Characteristics of abnormal sperm (Figure 2) were mainly categorised into two. The first category is sperm with head abnormalities, which are headless sperm, amorphous head sperm, flattened head sperm, banana head sperm, ring-shaped head sperm, and pinhead sperm. The second category is sperm with tail abnormalities which including double-tailed sperm, bent tail sperm, coiled tail sperm, and bent neck sperm (Sailer et al., 1997; Narayana et al., 2002; Kuriyama et al., 2005; Shetty, 2007; Demirci & Sahin, 2019).

Table 1:The reproductive organ weights (mean ± SEM) of male Sprague-Dawley rats in control (0 g/kg of bee bread), T1 (1 g/kg of bee bread), T2 (2g/kg of bee bread), and T3 (3 g/kg of bee bread) groups.

Mean weight (g/100g b.w.)	Control	T1	T2	T3
Testis	0.44 ± 0.02[a]	0.44 ± 0.02[a]	0.52 ± 0.03[a]	0.50 ± 0.02[a]
Epididymis	0.13 ± 0.01[a]	0.15 ± 0.01[a]	0.15 ± 0.01[a]	0.15 ± 0.01[a]
Seminal vesicles	0.06 ± 0.01[a]	0.07 ± 0.01[a]	0.07 ± 0.01[a]	0.07 ± 0.01[a]
Prostate	0.08 ± 0.01[a]	0.09 ± 0.01[a]	0.09 ± 0.01[a]	0.09 ± 0.01[a]

[a]superscript in the same row shows no significant difference ($p \geq 0.05$)

Table2: The sperm parameters (mean ± SEM) of male Sprague-Dawley rats in control (0 g/kg of bee bread), T1 (1 g/kg of bee bread), T2 (2g/kg of bee bread), and T3 (3 g/kg of bee bread) groups.

Sperm parameters	Control	T1	T2	T3
Sperm count ($X 10^6$)	19.17 ± 1.51[a]	27.67 ± 3.72[a]	28.17 ± 1.01[a]	29.33 ± 3.50[a]
Abnormal sperm (%)	15.42 ± 1.34[a]	10.67 ± 0.17[b]	9.25 ± 0.82[b]	8.17 ± 0.70[b]

[ab]superscripts in the same row shows a significant difference ($p<0.05$) b.w.: body weight

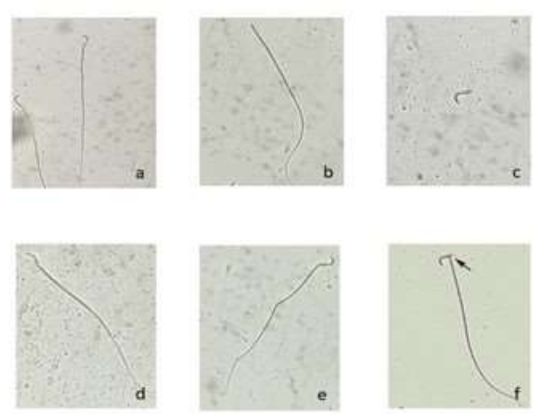

Figure2: Light microscopic image of the normal and abnormal sperm. a) normal sperm; b) headless sperm; c);tailless sperm d) hookless; e) bent tail sperm; f) bent neck sperm (400x).

DISCUSSION

There is no significant difference in the mean weight of male reproductive organs within all groups (Table 1). Testis weight was lower in the treatment groups compared to the control group. Previously, the consumption of honey caused no significant differences in weight, length, and width of testis between the control and treated groups (Syazana et al. 2011). Consumption of pollen caused no significant differences in male reproductive organs, including testis, epididymis, and seminal vesicle weights in the pollen groups. Furthermore, prostate gland weight increased significantly (Selmanoğlu et al. 2009).

The present result showed the percentage of abnormal sperm was significantly lower in the treated groups when compared to the control group. According to the previous study, the consumption of honey significantly increased normal sperm morphology as compared to the control group (Syazana et al. 2011). Besides, treated infertile men with Date pollen powder significantly increase in normal sperm morphology and sperm count after two months of treatment (Rasekh et al. 2015). Moreover, bee pollen is rich in B complex vitamins (thiamine, niacin, riboflavin, pyridoxine, pantothenic acid, folic acid, and biotin) and carotenoids (Mizrahi & Lensky,1997; Krell,1996; Schmidt & Buchmann 1992; Zafra 1979). Wong et al. 2002 reported that folic acid could improve sperm concentration but not sperm motility or sperm morphology.

Furthermore, the administration of Nigerian honey significantly increased sperm count and also normal sperm morphology (Oyelowo et al., 2014). Honey can interact positively with luteinising hormone, stimulates Leydig cells to produce more testosterone. Increased level of testosterone leads to the improvement of the sperm parameter. Testosterone plays an essential role in producing spermatogenesis, epididymal spermatozoa maturation & sexual desire (Rasekh et al. 2015). The presence of flavonoid components has a positive effect on sperm quality. The antioxidant can defend against oxygen stress, which altered sperm motility (Ceksterytë et al. 2006; Baltrušaitytė et al., 2007; Said et al. 2004; Sharma & Agarwal, 1996). Fructose in honey plays a vital role by providing energy, nutrients, and perfect alkaline medium for the sperm to maintain their viability and motility (Igbokwe et al. 2013). Honey could help in reducing the lipid peroxidation and oxidative stress on the sperm cells by providing antioxidant properties, which will react with reactive oxygen species (Erejuwa et al. 2012).

Bee bread is a mixture of honey, pollen, and bee secretions (Bakour et al. 2017). So, further study needs to be done to determine the effect of bee bread on the male reproductive system in rats. Thus, findings from the previous study may support that bee bread increased the percentage of normal sperm morphology as well as the male reproductive system in rats.

CONCLUSION

Currently, there is a lack of established research about the medicinal use of bee bread, especially in the male reproductive system of rats. From the present result, bee bread supplementation does not cause any adverse effect on the male reproductive system of rats. Furthermore, bee bread has a positive impact on sperm morphology. However, further research needs to be done to gain more information about the beneficial roles of bee bread on spermatogenesis, and its effect on testicular microstructure. It is also suggested that bee bread may be having a beneficial effect on sperm production if bee bread consumption is extended to a period longer than 28 days.

CONFLICT OF INTEREST

The authors declared that the present study was performed in the absence of any conflict of interest.

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AUTHOR CONTRIBUTIONS

MNH devised the project, the main conceptual ideas, and proof outline. FHZ contributed to the design and implementation of the research, to the analysis of the results and to the writing of the manuscript.

with these terms.

REFERENCES

Abdul-Ghani, A-S., Dabdoub, N., Muhammad, R., Abdul-Ghani, R., & Qazzaz, M. (2008). Effect of Palestinian Honey on Spermatogenesis in Rat. Journal Of Medicinal Food, 1-4.Apitherapy. Plenum Press, New York/London.

Bahmanpour, S., Talaei, T., Vojdani, Z., Panjehshahin, M.R., Poostpasand, A., Zareei, S. & Ghaeminia, M. (2006). Effect of phoenix dactylifera pollen on sperm parameters and reproductive system of adult male rats. Iranian Journal of Medical Sciences, 31(4), 208-212.

Bakour, M., Al-Waili, N.S., Menyiy, N.E., Imtara, H., Figuira, A.C., Al-Waili, T. & Lyoussi, B. (2017). Antioxidant activity and protective effect of bee bread (honey and pollen) in aluminum-induced anemia, elevation of inflammatory makers and hepato-renal toxicity. Journal of Food Science and Technology, 54(13), 4205–4212.

Baltrušaitytė V., Venskutonis P.R. & Čeksterytė V. (2007). Radical scavenging activity of different floral origin honey and beebread phenolic extracts, Food Chemistry, 101(2), 502-514.

Barene, I., Daberte, I. & Siksna, S. (2015). Investigation of bee bread and development of its dosage forms, Medicinos (teorija ir praktika), 21(1), 16-22.

Cekśterytë, V., Kazlauskas, S. & Racys, J. (2006). Composition of flavonoids in Lithuanian honey and beebread. Biologija, 2, 28-33.

Demirci, T. & Sahin, E. (2019). The effect of chronic stress and obesity on sperm quality and testis histology in male rats; a morphometric and immunohistochemical study. Histol Histopathol, 34, 287-302.

Gilliam, M. (1979). Microbiology of Pollen and Bee Bread : The Yeasts. Apidologie, 10(1), 43-53.

Haron, M.N., D'Souza, U.J.A., Jaafar, H., Zakaria, R. & Singh, H. J. (2010). Exogenous leptin administration decreases sperm count and increases the fraction of abnormal sperm in adult rats. Fertility and Sterility, 93(1), 322-324.

Haron, M.N., Wan, A.R.W.F., Sulaiman, S.A. & Mohamed, M. (2014). Tualang honey ameliorates restraint stress-induced impaired pregnancy outcomes in rats. European Journal of Integrative Medicine, 6(6), 657–663.

Igbokwe, V.U., Gege-Adebayo, G.I. & Samuel, O. (2013). Pure honey a potent fertility booster: activities of honey on sperm parameters in young adult rats. Journal of Dental and Medical Sciences. 9(6), 43-47.

Kieliszek, M., Pimowarek, K., Kot, M.A., Blazejak, S., Chlebowska-Smigiel, A., & Wolska, I. (2018). Pollen and bee bread as new health-oriented products: A review. Trends in Food Science & Technology, 71, 170-180.

Kuriyama, K., Kitamura, T., Yokoi, R., Hayashi, M., Kobayashi, K., Kuroda, J. & Tsujii, H. (2005). Evaluation of testicular toxicity and sperm morphology in rats treated with methyl methanesulphonate (MMS). The Journal of Reproduction and Development, 51(5), 657-667.

Makler, A. (1980). The improved ten-micrometer chamber for rapid sperm count and motility evaluation. Fertility and Sterility, 33(3), 337-338.

Mehraban, F., Jafari, M., Toori, A.M., Sadeghi, H., Joodi, B., Mostafazade, M., & Sadeghi, H. (2014). Effects of date palm pollen (Phoenix dactylifera L.) and Astragalus ovinus on sperm parameters and sex hormones in adult male rats. Iranian Journal of Reproductive Medicine, 12(10), 705-712.

Mizrahi, A., Lensky, Y. (Eds.), (1997). Bee Products: Properties, Applications and

Narayana, K., D'Souza, U.J. & Seetharama R.K.P. (2002). Ribavirin- induced sperm shape abnormalities in wistar rat. Mutation Research, 513(1-2), 193-196.

OECD (2008), Test No. 407: Repeated Dose 28-day Oral Toxicity Study in Rodents, OECD Guidelines for the Testing of Chemicals, Section 4.

Oyelowo, O.T., Adekunbi, D.A. & Dada, K.A. (2014). Original article protective role of Nigerian honey on sperm indices and testis in sucrose-fed rats. Bangladesh Journal of Medical Science, 13(02), 180–189.

Rasekh, A., Jashni, H.K., Rahmanian, K., & Jahromi, A.S. (2015). Effect of palm pollen on sperm parameters of infertile man. Pakistan Journal of Biological Sciences, 18(4), 196-199.

Rowe, P.J., Comhaire, F.H., Hargreave, T.B. & Mellows, H.J. (1993). WHO Manual for the standardized investigation and diagnosis of the infertile couple. Cambridge University Press, Cambridge.

Said, T.M., Agarwal, A., Sharma, R.K., Mascha, E., Sicca, S.C. & Thomas, A.J.Jr.(2004). Human sperm superoxide anion generation and correlation with semen quality in patients with male infertility. Fertility and Sterility, 82(4), 871-877.

Sailer, B.L., Sarkar, L.J., Bjordahl, J.A., Jost, L.K. & Evenson D.P. (1997). Effects of heat stress on mouse testicular cells and sperm chromatin structure. Journal of Andrology, 18(3), 294-301.

Schlegei, P.N. (1997). Is assisted reproduction the optimal treatment for varicocele-associated male infertility? a cost-effectiveness analysis. Urology, 49(1), 83-90.

Schmidt, J.O. & Buchmann, S.L., (1992). Other products of hive. In: Graham, J.M., Amgrose, J.T., Langstroth, L.L. (Eds.), The Hive and the Honey Bee: A New Book on Beekeeping which Continues the Tradition of ‘‘Langstroth on the Hive and the Honeybee’’. Dadant, Hamilton, pp. 928–977.

Selmanoğlu, G., Hayretdağ, S., Kolankaya, D., & Tüylü, A.Ö. (2009). The effect of pollen on some reproductive parameters of male rats. Pestic. Phytomed. (Belgrade), 24, 59–63.

Sharma, R.K. & Agarwal, A. (1996). Role of reactive oxygen species in male infertility. Urology, 48(6), 835-850.

Shetty A.J. (2007). The effect of gabapentin and phenytoin on sperm- morphology in wistar rats. Reproductive Biology, 7(3), 247-251

Syazana, N.S., Hashida, N.H., Majid, A.M., Sharifah, H.A.D. & Kamaruddin, M.Y. (2011). Effects of Gelam honey on sperm quality and testis of rat. Sains Malaysiana, 40(11), 1243–1246.

Urcan, A., Mărghitaș, L.Al, Dezmirean, D.S., Bobiș, O., Bonta, V., Mureșan, C.I. & Mărgăoan, R. (2017). Chemical composition and biological activities of beebread – Review. Bulletin UASVM Animal Science and Biotechnologies, 74(1), 6–14.

Vialli, K. (2014). Kyle Vialli - "Making Vitality Your Reality": Bee Bread & The Truth About Bee Pollen!. Downloaded from: http://kylevialli.com/bee-bread-the-truth-about-bee-pollen/. Accessed on 12 November 2017.

Wong, W.Y., Merkus, H.M., Thomas, C.M., Menkveld, R., Zielhuis, G.A. & Steegers-Theunissen, R.P. (2002). Effects of folic acid and zinc sulfate on male factor subfertility: a double-blind, randomized, placebo-controlled trial. Fertility and Sterility, 77, 491– 498.

Zafra, A.O., 1979. El polen em su salud. Florimiel, Puebla, Pue.

Zakaria, F.H. & Haron, M.N. (2018). Effects of bee bread on male reproductive system of sprague dawley rat. Journal of Agrobiotechnology, 9(1S), 12-17.