

Ethanol extract of dragon fruit and its effects on sperm quality and histology of the testes in mice.

Farid Abdul Aziz and Mahanem Mat Noor

School of Biosciences and Biotechnology, Faculty of Science and Technology, Universiti Kebangsaan Malaysia, 43600 Bangi, Selangor, Malaysia.

Abstract

The increasing prevalence of infertility cases is becoming a major public health problem in developing countries due to changes in diet and lifestyle. Dragon fruit (*Hylocereus costaricensis*-[HC]) plays an important role as a fertility agent due to its antioxidant and anti-proliferative properties. The main aim of the present study was to identify the effects of ethanol extract of HC on sperm quality and histology of the testes. A total of twenty 10-week-old male ICR mice were divided equally into four different groups. Ethanol extract of HC (diluted to 0.2 mL with distilled water) was gavaged for 25 days at a dose of 250 mg/kg, 500 mg/kg and 1000 mg/kg body weight to the animals of group II (n=5), III (n=5) and IV (n=5), respectively. The animals of group I (control, n=5) had distilled water (0.2 ml). Results were analyzed using one way ANOVA test and the data were significant at $p < 0.05$. Oral administration of HC extract showed an increased sperm count, sperm viability, and its production rate. The HC extract at a dose of 500 mg/kg was found to be most effective to increase sperm count whereas HC of 1000 mg/kg effectively increased sperm viability and its production rate. Histological examination of the testis showed high density of sperm in seminiferous tubules. From our present experimental findings we are tempted to suggest that the ethanol extract of HC could be a potential male fertility agent.

Key words: *Hylocereus costaricensis* (Dragon fruit), sperm count, sperm viability

Accepted August 14 2009

Introduction

The fruits of *Hylocereus* cacti, known as red pitaya have recently drawn much attention of growers worldwide, not only because of their red-purple colour and economic value as food products, but also for the antioxidant property of their betacyanin contents [1]. The *Hylocereus* genus, the climbing or epiphytic tropical American cacti with angular stems and mostly with white, fragrant, night blooming flowers. Pitaya, the fruit of some strains of this cactus family, native to the tropical forest regions of Mexico and South America is currently being grown commercially in Taiwan, Nicaragua, Colombia, Vietnam, Israel, Australia and USA [2].

Studies have shown that both the flesh and peel of the fruit are rich in polyphenols and also good sources of antioxidants [3]. Many investigators have moreover, reported that dragon fruits have a potent antioxidant activity. In fact, the red pitaya peel fulfills its promise to inhibit the growth of melanoma cells which suggests that it has antiproliferative effect and acts as an anti-cancer agent

[4]. More than 90% of male infertility cases are known to be due to low sperm quality [5]. In 30 – 40% cases of sperm abnormality, the main cause remains unknown (unexplained infertility) but the rest could be due to health problems such as chronic disease, lack of nutrition, genetic abnormality and environmental factors.

Reactive oxygen species (ROS) are highly reactive oxidizing agents (free radicals). ROS is formed at various organs including the testes. However, its inappropriate synthesis results in cellular DNA disintegration [6]. Antioxidants, in general scavenge and suppress the adverse effects of ROS and lipid peroxidation, hence the application of ROS scavengers is likely to improve the sperm function [7].

Although the presence of natural antioxidant and other vitamins in dragon fruit is proven to be beneficial for human health, yet no evidence has so far been documented about the effects of dragon fruit or its extracts on male fertility. Present study was therefore undertaken to identify whether the extract of *Hylocereus costaricensis*

shows any beneficial effects on sperm count, sperm viability and the rate of spermatogenesis

Materials and Methods

Preparation of *Holocereus costarisensis* (HC) extract

The dragon fruit (HC) was purchased from the local store, washed and peeled off. The flesh was then dried in an oven for two days at 50°C. Once dried, it was grinded and refrigerated at (4°C). Extraction process was carried out by using 95% denatured ethanol in Soxhlet machine, and then transferred into Rotowap machine to separate it from the ethanol. Samples were then placed in vials and were dried by using nitrogen gas.

Animal experimentation

All animal experiments were conducted according to the research protocols approved by UKMAEC Guidelines 2003 – Universiti Kebangsaan Malaysia Animal Ethics Committee. Ten-week-old ICR mice (*Mus musculus*) were procured from animal house of the Universiti Kebangsaan Malaysia. Animals were maintained on balanced diet and water *ad libitum*. A total of 20 mice with body weight ranging from 28 to 30 g were selected and randomly divided into four different groups, five in each group. Animals of Group I (controls) were force fed with distilled water. Animals of Group II identically received HC (250 mg/kg) extract while Group III and Group IV animals had 500 mg/kg and 1000 mg/kg of extract, respectively. The volume of each gavage was 0.2 mL (200 µL) and the procedure was repeated daily between 8.00-9.00 am for 25 consecutive days.

Sample collection

Animals were sacrificed using chloroform 24 hrs following the treatment schedule; testes and the liver were dissected out, cleared of their adhering tissue and weighed. Testes and the liver tissues were fixed in Bouin's solution for histological analysis.

Evaluation of sperm parameters

Sperm concentration from the cauda epididymis was determined with a hemacytometer using the method described by WHO [8]. The cauda epididymis was finely minced by anatomical scissors in 5 ml of Bigger-Whitten - Whittingham (BWW) medium [9] in a petri dish prior to incubation with 5% CO₂ for 30 min at 37°C to allow the migration of spermatozoa to the medium. For sperm morphology study, sperm suspension (10µL) was smeared on a glass slide and dried overnight at room temperature. The

slide was fixed with 100% methanol and finally stained with Giemsa diluted in PBS at 1:3 ratios. For viability study, sperm suspension (10µL) was placed on a slide and added with 10µL of Eosin and examined under the microscope at 400 X magnification. Heads of the spermatozoa which showed red coloration were considered to be mortal and vice versa.

Histological Analysis

To determine the changes in the testes and the liver, both tissues were fixed in a Bouin's solution for 24 hours, washed in running tap water, dehydrated through graded concentration of ethanol, embedded in paraffin wax, sectioned at 5 µm thicknesses and stained with Hematoxylin and Eosin (H&E). Testicular spermatogenesis ranging from germ cell production up to the stage of mature spermatozoa was evaluated. Histological status of the liver of the animals was also evaluated.

Statistical analysis

All data were subjected to a one-way analysis of variance (ANOVA) to determine the level of significance between control and the treated groups. A difference was considered significant at $p < 0.05$. Data are reported here as mean \pm standard deviation (\pm SD).

Results

Organ (testes and liver) weight

Administration of HC extract did not show any significant change in the weight of the testes and the liver (Table 1).

Table 1: Effects of ethanol extract of HC on organ weight.

Animal Group	Testes weight (g)		Liver weight (g)
	(L)	(R)	
Control	0.11±0.01	0.11±0.01	1.90±0.24
250 mg/kg	0.11±0.01	0.11±0.01	1.77±0.32
500 mg/kg	0.11±0.02	0.11±0.01	1.93±0.10
1000 mg/kg	0.11±0.02	0.11±0.02	1.81±0.11

L= left R= right

Cauda epididymis sperm quality

The effects of different doses of HC extract on sperm count, sperm viability and sperm morphology are presented in Table 2. The HC extract at 500 mg/kg significantly increased the sperm count ($p < 0.05$) while the dose of 1000 mg/kg significantly increased sperm viability ($p < 0.05$) compared to the controls. However, sperm morphology remained normal in all the groups of animals.

Table 2: Effects of ethanol extract of HC on sperm quality

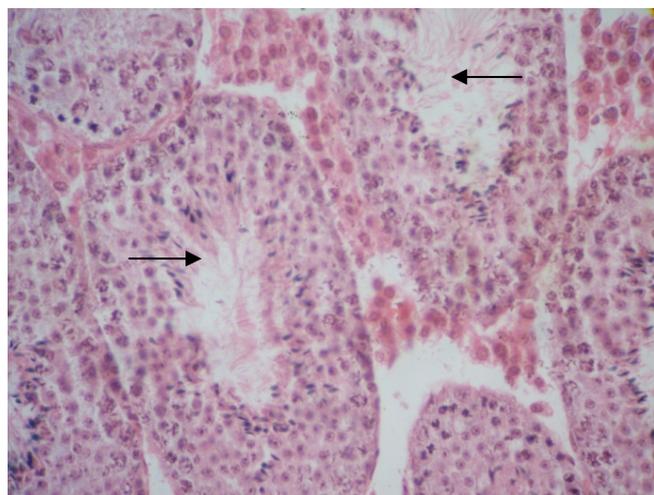
Animal Group	Sperm count ($\times 10^6$)	Sperm Viability (%)	Normal Morphology (%)
Control	13.5 \pm 2.47	36 \pm 0.09	87 \pm 0.01
250mg/kg	13.7 \pm 4.27	46 \pm 0.12*	89 \pm 0.02
500mg/kg	22.9 \pm 4.07*	51 \pm 0.07*	90 \pm 0.01
100 mg/kg	12.1 \pm 2.30	61 \pm 0.06*	92 \pm 0.02

Data are expressed as mean \pm S.D

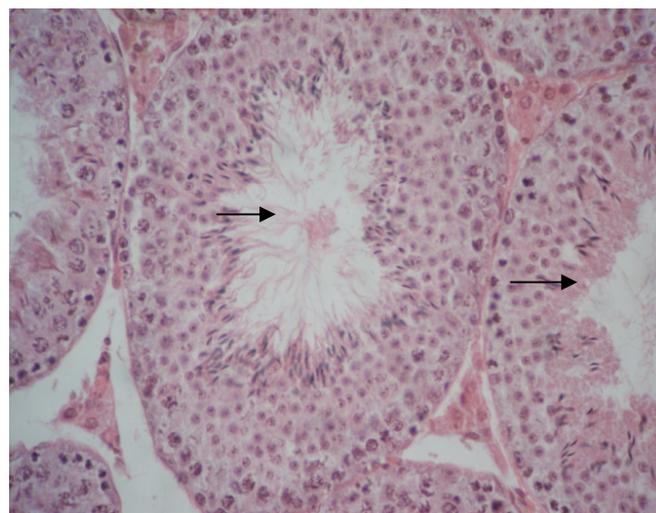
* $p < 0.05$

Effects of HC extract on spermatogenesis

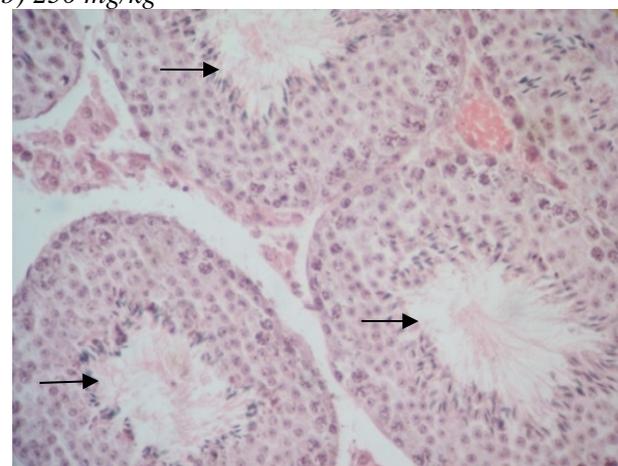
Seminiferous tubular structure suggested an increased tendency of spermatogenic process following the use of HC extract at a dose of 500 mg/kg which had further showed to be reduced following 1000 mg/kg (Fig. 1a, b, c, d).



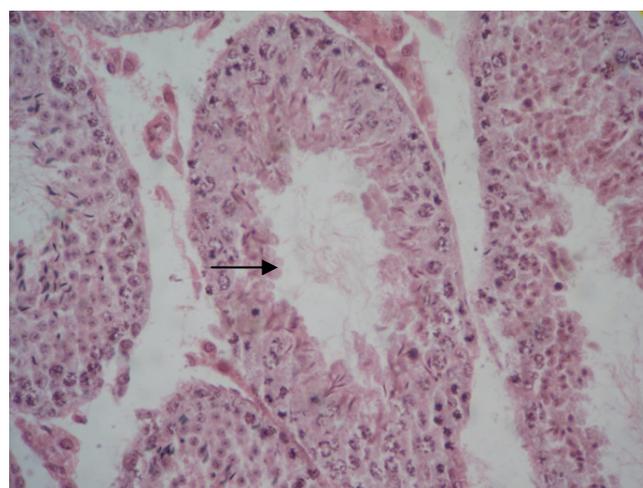
(a) Control



(b) 250 mg/kg



(c) 500 mg/kg



(d) 1000 mg/kg

Figure 1: Section of seminiferous tubules of mice from (a) control group and treated groups with (b) 250 mg/kg (c) 500 mg/kg (d) 1000 mg/kg of HC extract respectively, indicate the presence of spermatid in the lumen of testis (arrows). H&E (x 400)

Effects of HC extract on the liver

Histological structures of the liver of the treated groups did not differ from the controls. Neither any necrosis nor shrinkage of the hepatic cell nucleus was evident. Cytoplasm of the liver in the treated groups showed a flocculated structure similar to that found in controls, which indicates normal store of cellular glycogen, data not shown.

Discussion

Results of this study demonstrated that daily consumption of HC extract for 25 consecutive days increased the cauda epididymal sperm count and sperm viability.

HC is known to be rich in antioxidant such as ascorbic acid, betalains and polyphenols [10]. Besides, the oxygen radical absorbent capacity (ORAC), the antioxidant activity of HC was one of the highest compared to other tropical fruit. According to Chan & Babji [personal communication], among the antioxidant activity of extracts from various spices, ascorbic acid was used as a standard to compare the activity of diphenylpicrylhydrazyl (DPPH) for scavenging free radical from various spices extracted [11]. Hence, the ascorbic acid seems to be a very strong antioxidant that can effectively scavenge free radicals.

Previous studies explained that testicular cells are well equipped with enzymatic and low molecular weight antioxidants to maintain redox homeostasis and prevent the formation of reactive oxygen species (ROS) [12]. On the other hand, excessive formation of ROS may over-ride antioxidant defenses and results in oxidative stress-induced permanent damage to testicular cells. The high content of polyunsaturated fatty acids in testicular cell membrane easily undergoes lipid peroxidation and leads to oxidative stress [13, 14]. Oxidative stress consequently results in abnormal sperm production and function [15]. Therefore, ascorbic acid as a natural antioxidant could possibly be able to minimize generation of an excess ROS and subsequently protect the testicular damage [16].

The significant increase in the sperm count and viability at different dose levels of HC extract indicate that the levels of antioxidant may possibly increase with an increase in the dosage of HC extract and thus help to protect testicular germ cell and other spermatogenic cell death from oxidative stress [17]. However, further investigation is very much required to claim that the ascorbic acid content of HC extract is primarily responsible for increasing male fertility.

In conclusion, the resulting beneficial effects of the HC extract on different sperm parameters in mice may possibly

be mediated by preventing the formation of ROS in the testes.

Acknowledgment

The authors thank Prof. Dr. Amar Chatterjee from UiTM, Dr. Srijitt Das and Prof. Dr. Othman Omar from UKM for revising the paper. This work was supported by a grant from the School of Biosciences & Biotechnology, Faculty of Science & Technology, Universiti Kebangsaan Malaysia.

References

1. Wybraniec S, Mizrahi Y. Fruit flesh Betacyanin pigments in *Hylocereus cacti*. *J Agri Food Chem* 2002; 50: 6086-6089
2. Mizrahi Y, Nerd A, Nobel PS. *Cacti as Crops*. *Horti Rev* 1997; 18: 291-320
3. Wu LC, Chen YC, Ho JA, Yong CS. Inhibitory effect of red koji extracts on mushroom tyrosinase. *J Agri Food Chem* 2003; 51: 4240-4246
4. Li CW, Hsiu WH, Yun CC, Chih CC, Yu IL, Ja-an AH. Antioxidant and Antiproliferative activities of red Pitaya. *J Agri Food Chem* 2006; 95: 319-327.
5. Simon H. *Infertility In Men*. Harvard Medical School Press, Harvard 2006.
6. Sikka SC. Oxidative stress and role of antioxidants in normal and abnormal sperm function. *Front Biosci* 1996; 1: 78-86.
7. Vernet P, Aitken RJ, Drevet JR. Antioxidant strategies in the epididymis. *Mol Cell Endocrinol* 2004; 216: 31-39
8. World Health Organization. WHO laboratory manual for the examination of the human semen and sperm-cervical mucus interaction. Cambridge University Press, Cambridge 1999.
9. Biggers JD, Whitten WK, Whittingham DG. The culture of mouse embryos in vitro. In: *Methods In Mammalian Embryology*. Daniel JC (Ed) Freeman, San Francisco. 1971; 86-116.
10. Li CW, Hsiu WH, Yun CC, Chih CC, Yu IL, Ja-an AH. Antioxidant and antiproliferative activities of red Pitaya. *J Agri Food Chem* 2006; 95: 319-327.
11. Silva BC, Dias ACP. Evaluation of the free radical scavenger of *Hyperriceum perforatum* alcoholic extracts. *Rev de Fito* 2002; 2 (1): 133.
12. Bauche F, Fouchard M, Jgou B. Antioxidant system in rat testicular cells. *J Reprod Toxicol* 1994; 349: 392-396.
13. Alvarez JG, Touchstone JC, Blasco L, Storey BT. Spontaneous lipid peroxidation and production of hydrogen peroxide and superoxide in human spermatozoa. Superoxide dismutase as a major enzyme protectant against oxygen toxicity 1987; 8: 348-388.
14. Koizumi T, Li ZG. Role of oxidative stress in single dose, cadmium-induced testicular cancer. *J Toxicol Env* 1992; 37: 25-36.

15. Shen H, Ong C. Detection of oxidative DNA damage in human sperm and it's association with sperm function and male fertility. *Free Radic Med Biol* 2000; 28: 529-536.
16. Yousef MI, Abdallah GA, Kamel KI. Effect of ascorbic acid and vitamin E supplementation on semen quality and biochemical parameters of male rabbits. *J Anim Reprod Sci* 2003; 76: 99-111.
17. Hew KW, Heath GL, Jiwa AH, Welsh MJ. Cadmium in vivo causes disruption of tight junction junctions-associated microfilaments in rat sertoli cells. *J Reprod Biol* 1993; 49: 840-849.

Correspondence:

Mahanem Mat Noor

School of Biosciences and Biotechnology

Faculty of Science and Technology

Universiti Kebangsaan Malaysia, 43600 Bangi, Selangor

Malaysia

E-mail: mahanem@ukm.my

Effects of dragon fruit extract on sperm quality.....