ORIGINAL ARTICLE

Morphine and *Phoenix dactylifera* (dates) effects on the histological features of male rat reproductive organs

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Background: Previous studies have shown that morphine negatively effects male fertility while *Phoenix dactylifera* (dates) could cure male infertility by the exhibition of antagonist effects. This study was conducted to assess the possible ameliorating effects of dates on the histological features of morphine-induced male rat reproductive organs. **Materials and Methods:** Adult male Sprague Dawley rats age 7–9 weeks old, 200–250 g body weight (BW) were divided into six rats per each group: Group 1, force-fed with distilled water, 1 ml/kg BW for 35 days (control); Group 2, intramuscularly (IM) injected with morphine, 20 mg/kg BW for 7 days followed by force-fed with distilled water for 28 days; Group 3, force-fed with distilled water for 7 days followed by crude *P. dactylifera* extract, 200 mg/kg for 28 days; Group 4, injected (IM) with morphine, 20 mg/kg BW for 7 days followed by force-fed of crude *P. dactylifera* extract, 200 mg/kg for 28 days. Rats were sacrificed on day 36. The seminal vesicle (SV) and prostate gland (PG) were removed and fixed before histological processes. **Results:** In morphine-treated rats, the SV showed the absence of honeycomb-like appearance with flattened columnar cells while in the PG, eosinophilic secretion was noted to be absent from glandular lumina as compared to the control group. Administration of *P. dactylifera* extract in Group 4 showed improvement in histoarchitecture of the SV and PG with complex mucosal infoldings and glands luminal filled with secretion. **Conclusion:** *P. dactylifera* extract has a protective effect against the adverse effects of morphine on the male rat reproductive organs.

Key words: Dates, morphine, *Phoenix dactylifera*, prostate gland, rat, seminal vesicle

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INTRODUCTION

In Malaysia, the number of drug abusers has increased from 15,101 (2010) to 21,777 (2014).[1] Based on the National Anti-Drugs Agency, 96.79% of the illicit drug users are men and most commonly, abused drugs are opioid, which refers to heroin and morphine.[1] Morphine is an opioid pain medication that acts on the central nervous system (CNS) for strong analgesic action. Despite its beneficial use, it has some adverse side effects such as drowsiness, vomiting, constipation, and hormonal imbalance. Long term used of morphine causes oxidative damage to the liver, kidney, and brain.[2] In addition, excessive or repeated use of the drug may play a significant role in male infertility.[3,4] Several studies have shown that chronic morphine exposure adversely affected male fertility by reducing testosterone, luteinizing hormone (LH) and follicle-stimulating hormone (FSH) levels as well as decreasing the partial weights of testes, seminal vesicles (SVs), and prostate glands (PGs).[5,6]

Columnar epithelial cells are the crucial part in the SV and PG as their functions are to secrete fructose and prostaglandins into their glandular lumina, which provides energy for sperm. Therefore, the decreased weight of the SV and PG could be due to the reduction

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in the height of columnar epithelial cells and secretion in the lumina of the glands.[7,8] Similarly, Londonkar *et al*.[9] found that morphine caused a reduction in the weight of epididymides and vas deferens. As a result of its gonadal activities inhibition, it also lowered the sperm count in the cauda epididymides. Through these studies, the authors suggested the main cause for the chronic morphine adverse effects was through hypothalamo-hypophyseal-gonadal axis that would lead to long-term endocrine disturbances during sexual maturation.[10] Due to these findings, therapeutic agents such as gonadal stimulating drugs to induce hormonal functions have been used as treatment for male infertility.

Traditional medicines are widely used and aggressively been studied by researchers as they are cheap and locally available. These medicines have minimum side effects as compared to modern medicines.[11,12] One of the fruits that have long been used as a therapeutic agent is dates (*Phoenix dactylifera*) which have potential in treating chronic toxicity of opioids such as morphine.[13] El-Kott *et al*.[14] reported that *P. dactylifera* extract possesses anti-oxidant and anti-infertility effects on male rats as it comes with various sources of vitamins and energy. Previous studies also confirmed that *P. dactylifera* contains cholesterol, carotenoid, and gonad-stimulating components that could stimulate gonadotropin activity.[15,16] The stimulation of gonadotropin-releasing hormone (GnRH) would increase the production of LH, FSH, and testosterone, thus improve the function of the male reproductive system. *P. dactylifera* suspensions were also noted to increase the weight of testis and epididymis as well as enhance the sperm motility, count, morphology, and its DNA quality.[17]

Even though it has been well established that *P. dactylifera* has ameliorating effects on male reproduction, to the best of our knowledge, the effects of *P. dactylifera* on morphine-induced infertility has not yet been examined. Therefore, the present study was conducted to observe such effects of *P. dactylifera* on the reproductive organs, the SV and PG, of male rats after morphine administration.

MATERIALS AND METHODS

Preparation of extract

The *P. dactylifera* fruits were purchased from a local market. The fruits flesh were boiled with distilled water and pulverized using an electric blender. It was then filtered and freeze-dried to remove the water content until it became powder and kept in-20°C freezer. On usage, the powder was diluted with distilled water and stored in a refrigerator at 4°C to avoid any contamination. This solution was given to the rats at a dose of 200 mg/kg of body weight (BW).[18]

Drug

Morphine solution was obtained from the pharmacy of the University Malaya Medical Centre (UMMC). It was used as a stock solution to treat the rats at a dose of 20 mg/kg of BW.[19]

Animal housing

Experimental procedures were conducted based on the Guideline for Animal of the Institutional of Animal Care and Use Committee, University of Malaya [PASUM/30/12/2015/AB (R)]. Twenty-four male Sprague – Dawley rats (7–9 weeks) 200–250 g were obtained from UMMC, University of Malaya. The rats were reared in the animal house at the Centre for Foundation Studies in Science, University of Malaya (Pusat Asasi Sains Universiti Malaya, PASUM). The animals were acclimatized for a week before experiment. Two rats were housed per cage with sawdust as bedding under standard laboratory condition, 27°C room temperature with good ventilation. Light and dark periods were approximately 12 h/day, respectively. Chow food and tap water were given to the rats' *ad libitum* for 35 days throughout the experimental periods.

Experimental design

Animals were grouped into four equal groups (6 rats each): (i) Group 1 was force-fed with distilled water, 1 ml/kg BW for 35 days, (ii) Group 2 was intramuscularly (im) injected with morphine, 20 mg/kg BW for 7 days followed by force-fed with distilled water for 28 days, (iii) Group 3 was force-fed with distilled water for 7 days followed by crude *P. dactylifera* extract, 200 mg/kg for 28 days, and (iv) Group 4 was injected (im) with morphine, 20 mg/kg BW for 7 days followed by force-fed of crude *P. dactylifera* extract, 200 mg/kg for 28 days. Rats were sacrificed on day 36 by overdose of ketamine-xylazine injection (im).

Preparation of histological study

The SVs and PGs were extracted, fixed in Davidson's solution for 2 days and then stored in formalin solution (Cat no: K46046503441, Merck) prior subjected to histological processes. The tissues were dehydrated with a series of graded alcohol solutions. The dehydrated tissues were then infiltrated and embedded in a small block of paraffin wax (Paraplast, USA) before sectioning at 5 micrometer (μm) thickness by using a microtome (Cat no: 08050282, Feather). The tissue sections were then mounted on the microscope slides. This was followed by the tissue clearance process in xylene (Cat no: 1330-20-7, Systerm) and staining with hematoxylin and eosin solution. A mounting medium, dibutyl phthalate polystyrene xylene (Cat no: 100579, Merck) was used to adhere to the tissue on the slide with the coverslip. The slides were then observed under light microscope (Olympus, Japan) with ×20 and ×40.

Histological study

General histoarchitecture of the seminal vesicle and prostate gland

The SV and PG were analyzed under light microscope for the general histoarchitecture. The normal SV consists of convoluted folds of mucosal layers which form the honeycomb-like appearance. The mucosal layer is made up of lamina propria and pseudostratified columnar cells. It is surrounded by outer longitudinal and inner circular layers of smooth muscles. The lumina are filled with acidophilic secretion. On the other hand, the mucosa of normal PG consists of lamina propria and two layers of epithelial tissue that are the outer layer of the low cuboidal and inner layer of tall columnar epithelia. The papillary infoldings of these layers are distinguished with the presence of glandular lumina for its eosinophilic secretion.[20,21]

Measurement of the columnar epithelial cells of the seminal vesicle and prostate gland

The SV and PG were analyzed under light microscope using NIS-Elements Imaging System Software (Nikon Corporation, Minato-ku, Tokyo 108-6290, Japan). The height of the columnar epithelial cells (length along the apical-basal axis) was measured in μm under × 40 for SV and ×20 for PG.[7]

Statistical analysis

Data analysis was performed with Statistical Package for Social Science (SPSS) Version 23.0 (IBM Corporation, Armonk, New York, U.S.) and the results were expressed as mean ± standard error the variables were compared using one-way ANOVA test and Duncan multiple range test $P < 0.05$ was considered statistically significant.

RESULTS

Histology of the seminal vesicle

Control group

The histoarchitecture of SV in this group exhibited highly folded mucosal layers with tall pseudostratified columnar cells which form the honeycomb-like appearance. The lumina were filled with acidophilic secretion [Figure 1a].

Morphine group

The morphine group showed an absence of the honeycomb-like appearance. Columnar cells appeared to be flattened with the absence of acidophilic secretion in the glandular lumina as compared to the control group [Figure 1b].

Phoenix dactylifera extract group

Histological appearance of SV in this group was similar to that of in the control group with the presence of the honeycomb-like appearance. The gland was lined by pseudostratified columnar cells, and its acidophilic secretion was distinguished in the lumina [Figure 1c].

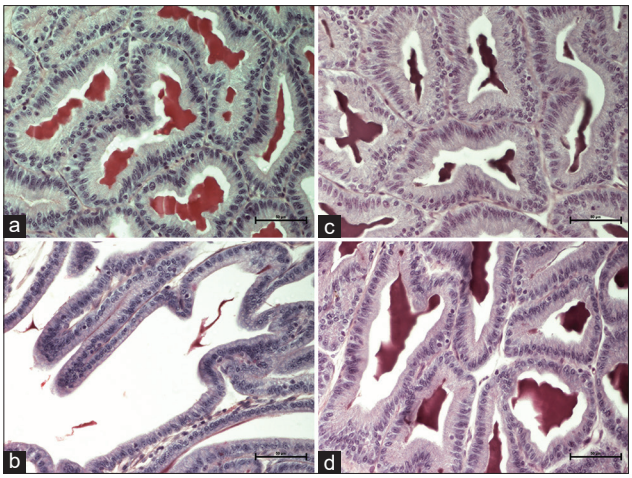

Figure 1: Photomicrograph of transverse section of rat's seminal vesicle. (a) Control group showed normal columnar cells lining the honeycomb-like appearance with acidophilic secretion filled the lumen. (b) Morphine-treated group showed flattened columnar cells with the absence of honeycomb-like appearance and acidophilic secretion. (c) *Phoenix dactylifera* extract group and (d) Morphine and *Phoenix dactylifera* extract group showed improvement in the histoarchitecture of the glands with tall columnar cells lining the honeycomb-like appearance and the acidophilic secretion fill up the lumen

Morphine - Phoenix dactylifera extract group

The presence of highly folded mucosal layers with tall pseudostratified columnar cells was observed in this group as compared to the morphine group. The honeycomb-like appearance was noted with acidophilic secretion filled lumina. There was the improvement of the gland histoarchitecture in this group as compared to that of in the morphine group [Figure 1d].

Histology of the prostate gland

Control group

In this group, the PGs were noted to have tall columnar and basal cells with normal papillary infoldings. The glandular lumina were filled with eosinophilic secretion [Figure 2a].

Morphine group

This group showed the absence of papillary infoldings of the columnar cells, which appeared to be flattened as compared to the control group. The eosinophilic secretion was also absent in the glandular lumina [Figure 2b].

Phoenix dactylifera extract group

The papillary infoldings of the epithelium into the lumina with the eosinophilic secretion were clearly seen in this group similar to that of found in the control group [Figure 2c].

Morphine - Phoenix dactylifera extract group

Complex papillary infoldings of the tall columnar cells were observed in this group. The glandular lumina were filled

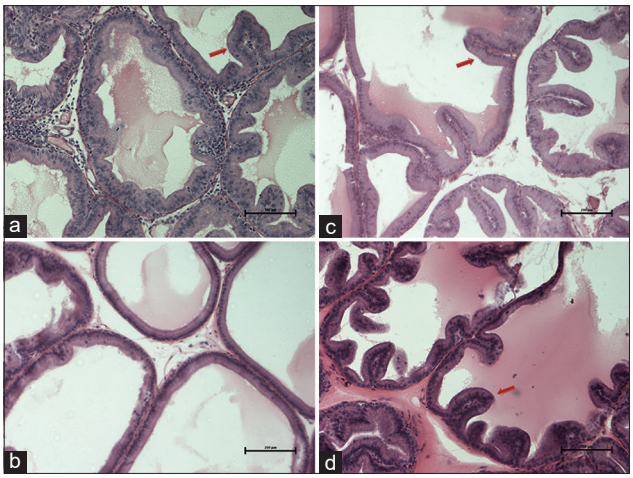

Figure 2: Photomicrograph of transverse section of rat's prostate gland. (a) Control group showed a normal papillary infoldings of the mucosa (red arrow) into the lumina that was filled with eosinophilic secretion. (b) Morphine-treated group showed the absence of papillary mucosal infoldings with the flattened columnar cells and empty ductal lumina (c) *Phoenix dactylifera* extract group and (d) Morphine and *Phoenix dactylifera* extract group showed complex mucosal infoldings with eosinophilic secretion in the lumina

Table 1: Mean square analysis of variance for the columnar epithelial cell height of seminal vesicle and prostate gland

Source of variation	Df	Mean square Columnar epithelial cell height (μm)	
		Seminal vesicle	Prostate gland
Treatment	3	630.22*	436.61*
Error	188	14.44	13.69
Total	192		

*Significant difference at $P<0.05$

Table 2: Height of the columnar epithelial cells of seminal vesicle and prostate gland in rats treated with morphine and *Phoenix dactylifera* extract ($n=6$)

Group	Parameters (μm), mean±SE	
	Seminal vesicle	Prostate gland
Control	20.98±3.90^{ab}	26.36±3.64^{b}
Morphine	14.56±2.76^{a}	20.09±3.59^{a}
P. dactylifera	23.00±4.54^{b}	25.84±3.50^{b}
Morphine - *P. dactylifera*	20.32±3.79^{ab}	26.13±4.04^{b}

^{a,ab}Superscripts in the same column show significant difference at $P<0.05$.
P. dactylifera=Phoenix dactylifera; SE=Standard error

with eosinophilic secretion as compared to that of in the morphine group [Figure 2d].

Height of columnar epithelial cells in the seminal vesicle

The analysis of variance indicated that the treatments had significantly affected the height of columnar epithelial cells in all groups ($P < 0.05$) [Table 1]. The morphine group showed the significant lowest mean height of columnar epithelial cells (14.56 ± 2.76 μm) among all groups ($P < 0.05$). The highest mean height of columnar epithelial cells was observed in the *P. dactylifera* group (23.00 ± 4.54 μm) as compared with the other groups ($P < 0.05$) [Table 2].

Height of columnar epithelial cells in the prostate gland

The analysis of variance showed that the treatments had significantly affected the height of the columnar epithelial cells in all groups ($P < 0.05$) [Table 1]. The height of columnar epithelial cells in the morphine group was the lowest (20.09 ± 3.59 μm) among all groups ($P < 0.05$) [Table 2].

DISCUSSION

Morphine has long been used as a medicinal treatment for its analgesic action. Many studies reported by researchers had shown that exposure to morphine increased the percentage of infertility among males.[22,23] In the present study, nonexistence of acidophilic secretion in the lumen with the absence of honeycomb-like appearance was observed in the SV of rats treated with morphine. Similarly, deficiency of eosinophilic secretion in the glandular lumina with less papillary infoldings of the columnar epithelial cells of the PGs was also noted in the same group of rats.

In addition, the height of the columnar epithelial cells of SV and PG of the rats was significantly reduced compared to other treatment groups. These findings were found to be concurrent with previous studies that showed similar adverse effects of morphine on the SV and PG.[4,24,25]

It was revealed that illicit drugs like morphine could cause damage to the structural integrity of the secondary sex organs by inhibition of the LH secretion, which subsequently leads to the reduction of testosterone levels.[25,26] Adams *et al*.[6] had shown that morphine reduced the LH and FSH as well as the testosterone by disrupting the GnRH in the pituitary glands which is essential for androgen synthesis. This was supported by another study which revealed that structural changes found in the secondary sex organs were caused by alteration in the gonadal and pituitary functions.[21,27] Therefore, this could be the reason for the disruption of SV and PG structures found in the present study since the development and function of the secondary sex organs are dependent on the production of the hormones.

Various medicinal plants, including dates fruits, were recommended for the treatment of various diseases as ingredients found in them possess anti-oxidant, anti-inflammatory, and antibacterial activities.[28] The therapeutic effect of *P. dactylifera* as a traditional medicine for male infertility had been shown by various studies conducted in the past few decades.[17,29,30] Similar findings were observed in the present study where *P. dactylifera* given to rats induced morphine have shown an improvement in the histoarchitecture of the SV and PG. Both glands have shown highly folded mucosal layers with a significant increase in the height of their epithelia. There was also the presence of acidophilic secretion in the lumina of the glands.

Previous studies reported that the gonadotrophin like substances or steroidal compound presence in dates palm pollen would increase the gonadotropin activity in the pituitary glands of the rat.[15,16,31] Stimulation of GnRH in the pituitary glands would increase the level of androgenic hormone such as testosterone levels in the male reproductive system.[32,33] Since the SV and PG are hormone-dependent organs for its function and development, an increase in the androgen synthesis might be the reason for the improvement in the histoarchitecture of the SV and PG in *P. dactylifera* group of the present study.

In comparison to other fruits, dates were found to contain extremely high levels of phenolics, which was hypothesized to have been formed as a result of exposure to extreme temperature and climate.[34] Plant polyphenols have been found to possess a wide range of biological effects such as estrogenic and anti-estrogenic activity, anti-proliferative activity, induction of cell cycle arrest and apoptosis, prevention of oxidation, regulation of the host immune system, anti-inflammatory activity, up-regulation of genes producing anti-oxidant enzymes, and the ability to change cellular signaling.[35] Dates with its anti-oxidant properties have the capacity to act as potent scavengers of reactive oxidative species. This would allow the body to sustain or recover its normal levels of endogenous enzymes such as catalases and peroxidases that protect the body at a cellular level from any toxicants exposure.

In addition, aqueous dates extract also possesses potent free radical scavenging activity. Aqueous *P. dactylifera* extract of 0.8 mg/mL was shown to scavenge 50% of superoxide radicals formed by photoreduction of riboflavin and 100% of superoxide radicals at 1.5 mg/mL.[36] This beneficial factor could also contribute to ameliorating the damage to the epithelial cells of the mucosal layers in the SV and PG of morphine-induced rats, as seen in the present study.

Based on the findings, the present study was the only study that focused on the potential healing effects of *P. dactylifera* against the adverse effects of morphine on the SV and PG. However, there were a few limitations in this study. First, only a single dosage of morphine (20 mg/kg) was used, whereas drug users might be using higher dosages of morphine. Thus, higher multiple dosages of morphine could be used to strengthen this study. Second, the duration of morphine exposure in this study was limited to only 7 days, which might not reflect the longer duration used by drug users as a result of the drug's addiction. As for the future study, we suggest investigating the hormonal levels, which are crucial for structural development and functioning of the male reproductive organs. Further study should also explore the underlying healing mechanism of *P. dactylifera.*

CONCLUSION

It is apparent that *P. dactylifera* exerts healing effects on the morphine-induced male rats' reproductive organs. These beneficial effects of *P. dactylifera* led to the improvement in the tissue histoarchitecture and function of columnar epithelial cells in the SV and PG. This study supports the use of an inexpensive dietary supplement such as the *P. dactylifera* extract in improving male fertility among morphine addicts.

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Conflicts of interest

There are no conflicts of interest.

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