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AMELIORATIVE EFFECT OF *Phoenix dactylifera* EXTRACT ON MORPHINE-INDUCED DAMAGE OF SPERM QUALITY AND TESTICULAR HISTOLOGY IN RATS

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ABSTRACT

Forty Sprague-dawley male rats were divided into 4 groups; Control, rats were forced feed with distilled water, 35 days; Morphine, rats were intramuscularly injected with morphine (20 mg/kg), 7 days; *P. dactylifera* (date), rats were forced feed with date aqueous extract, 28 days, and Morphine-*P. dactylifera*, rats were injected with morphine (20 mg/kg), the first 7 days and forced feed with date aqueous extract, another 28 days. The testis and epididymis were harvested for histological and sperm parameter analysis. The Morphine- *P. dactylifera* group showed significantly higher sperm count ($109.30 \pm 5.01 \times 10^6$ sperm/ml) and motility ($72.80 \pm 4.48 \times 10^6$ sperm/ml) than the morphine group ($P < 0.05$). Significantly higher normal and lower abnormal sperm were observed in the Morphine- *P. dactylifera* group in comparison to the Morphine group. Higher in life sperm (90.50 ± 0.00 %) and lower in dead sperm (9.50 ± 0.00 %) were also observed in the Morphine- *P. dactylifera* group than the Morphine group. Testicular cells were found to be significantly higher in Morphine- *P. dactylifera* group as compared to the Morphine group. These research findings have provided additional information on the beneficial effect of *P. dactylifera* to ameliorate the damages caused by morphine on sperm parameters and testicular histoarchitecture. Thus, this study may suggest the potential use of *P. dactylifera* as a supplement to improve male reproductive functions.

Keywords: *Phoenix dactylifera*, morphine, sperm, testis, rats.

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INTRODUCTION

Infertility can be defined as the inability of a couple to achieve pregnancy with unprotected intercourse after a year or more. Infertility is a major health problem worldwide and is estimated to affect 8–12% of couples in the reproductive age group (Agarwal *et al*., 2020). The Global Burden of Disease database for 195 countries during 1993-2017 on primary and secondary infertility prevalence rate (PSIPR) reported lower among men than women (Borumandnia *et al*., 2022). Males are found to be the only responsible for 20-30% of infertility cases and contribute to 50% of overall cases (Agarwal *et al.*, 2015). Lifestyle activities and environmental contaminant exposures contribute as significant risk factors for male infertility and poor fertilization capacity of the oocyte (Leisegang and Henkel, 2020). Excessive exposure to heat, radiation, chemicals, caffein, tobacco smoke and recreational drugs could be the reason for hormones disruption and failure of the reproductive organ to function optimally lead to male infertility (Durairajanayagam, 2018).

Cytotoxic drugs and other drugs are potentially affect men's fertility through various mechanism. Drug used in pharmacological treatments have negative impact on male fertility such as spermatogenesis disruption, decrease in sexual function and sperm maturation (Semet *et al.*, 2017). It causes possible alterations in steroidogenesis and spermatogenesis by interfering with testosterone production (Duca *et al.*, 2019). Results from previous studies have shown that drug abused contributes to damage of hypothalamic-pituitary-gonadal functions, lower sperm concentration, detrimental effect on testicular structure, increased sperm DNA fragmentation and apoptosis resulting in male infertility (Fronczak *et al.*, 2012; Ajayi and Akhigbe, 2020).

Morphine is an opioid pain reliever which produces its effects by affecting a subset of neurons responsible for pain sensitivity. Morphine either exogenous or endogenous may modulate gonadal functions via their interference with hypothalamic-pituitary-gonadal (HPG) axis (Jamshidian *et. al.*, 2019). Previous research has shown that exogenous morphine reduces the fertility of male opioid abusers, as did male

patients who used morphine for pain control (Drobnis and Nangia, 2017; Sansone *et. al.*, 2018). Disruption of the HPG axis causes a failure of the testes to produce adequate levels of testosterone and a normal number of sperm (Yibrah *et al.*, 2019). Concurrently, previous findings indicated that exposure to morphine decreased the histological parameters, germinal thickness, count, viability, morphology and motility of sperms along with significant reduction of testosterone, LH and FSH (Jalili *et al.*, 2016; Roshankhah *et al.*, 2017; Salahshoor *et al.*, 2018). Administration of morphine to the opioid system significantly reduces sex hormone levels, spermatogenesis, and adult sperm count in male rats (Ahmadnia *et al.*, 2016). A review by Antony *et al.* (2020) stated that the opioid drug therapy could induce hypogonadism both in males and females, depending on the concentration of the drug and duration of therapy.

Complementary therapies for infertility have received growing attention during recent years and various antioxidants, nutritional approaches, and medicinal plants have been proposed for the treatment of fertility problems in infertile and sub fertile couples (Abdi *et al*., 2017). Several medicinal plants with antifertility or fertility boosting effects have been traditionally used to either decrease or increase male fertility throughout the world. Fertility-related properties of these plants have also been the subject of interest in modern scientific research (Jain *et al*., 2015). Following men's increasing interest in effective herbal treatments of infertility, complementary approaches to infertility treatment have received growing attention (Yao and Mills, 2016).

Traditional methods using herbal medicines derived from plants have been used to improve male fertility (Gamit *et al.*, 2022). *Anacyclus pyrethrum* root alcoholic extract administration to albino rats would stimulate the hypothalamus-adenohypophysis-gonad axis and increase sperm count, motility, serum FSH, LH, and testosterone (Shahraki *et al*., 2019). Another study reported that treatment of *Withania somnifera* to wistar rat would increase testosterone and cholesterol, spermatozoa and seminiferous tubules diameter (Gamit *et al*., 2020). Alkaloidal fraction of *Argyreia speciosa* root was discovered by Vyas *et al*. (2020) to increase concentration of testosterone and sperm counts. The authors also reported an increase in seminiferous tubules diameter with high numbers of spermatozoa found in the tubule. The fraction could also elevate serum testosterone levels in the treated animals.

Date, *Phoenix dactylifera* of the family Arecaceae, is a rich source of antioxidants. The antioxidant activity of dates is indicated by the presence of phenolic compounds, flavonoids, and Vitamin C (Abdelaziz and Ali, 2014). Tauqeer *et al.* (2014) discovered that pollens and fruits of the date palm, have the potential to cure infertility in male and female by increasing the levels of estrogen and testosterone. The administration of ethanol extract of date gave anti-infertility activity to mice, which was characterized by an increase in the number of spermatozoa, the percentage of sperm motility, and weight of the testis (Dillasamola *et al.*, 2019). In line with other studies, Zare *et al.* (2020) demonstrated a significant protective effect of the date fruit extract on sperm parameters and testicular changes in male mice testis-induced formaldehyde toxicity. Dates fruit extract showed tremendous ability to enhance sperm motility as it consistently reduced sperm abnormality (Ubah *et al.*, 2021). Atoigwe-Ogeyemhe *et al.* (2018) reported that the incidence of sexual abnormalities is on the rise and thus, require a faster approach, which is cost effective and without side effects. Therefore, the present study was conducted to evaluate the potential healing properties of date palm on damaging effect of morphine-induced rat sperm and testicular cell count.

MATERIALS AND METHODS

Maintenance of Rats: Forty male adult Sprague-Dawley rats (7 - 9 weeks old, 200 – 250 grams) were obtained from the animal house of University Malaya Medical Centre (UMMC), University of Malaya and were randomly divided into 4 different groups; Control, *P. dactylifera*, Morphine, and Morphine- *P. dactylifera*.

Rats were kept in the animal house at the Centre for Foundation Studies in Science, University of Malaya. The rats were allowed to acclimatize for two weeks prior to treatment with several conditions respects to their needs. The room was maintained at the temperature of 22^{o}C to 26^{o}C and equipped with 12 hours light and dark, alternately. All rats were provided with sufficient pellet 20 - 30 grams and water *ad libitum* daily. The sawdust bedding was changed once a week to ensure a hygienic environment for the rats. The rats were weighed once every three days.

Preparation of *Phoenix dactylifera* Aqueous Extract: Imported *Phoenix dactylifera* was purchased from the local store at Shah Alam, Selangor, Malaysia. Dried fruits, 50 grams were boiled in distilled water, 200 mL for 30 minutes. The boiled samples were filtered and concentrated to approximately 60% of its initial volume on a hot plate. The concentrated liquid was freeze-dried using Laboratory Freeze Dryer (Telstar LyoQuest) at -55^{o}C to yield the date aqueous extracts powder and were stored at -20^{o}C prior to use. The powder was diluted with distilled water for 50 mg/mL as stock solution and kept in refrigerator at 4^{o}C for a week.

Treatment group: Rats were forced feed with distilled water (1 mg/kg bodyweight - BW) for 35 days for Control group, intramuscularly injected with Morphine (20 mg/kg BW) for 7 days for Morphine group, forced feed with *P. dactylifera* extract (200 mg/kg BW) for 28

days for *P. dactylifera* group, and intramuscularly injected with Morphine (20 mg/kg BW) for 7 days prior to force fed with *P. dactylifera* aqueous extract (200 mg/kg BW) for 28 days. All rats were sacrificed on day 36. The testis and epididymis were harvested for histological studies and sperm parameter evaluation, respectively. The experiment was performed in accordance with Guideline for Animal of the Institute of Animal Care and Use Committee (IACUC), University of Malaya [PASUM/30/12/2015/AB(R)].

Sperm parameter: The cauda epididymis was harvested and transferred into 10 mL of Toyoda Yokoyama Hosi (TYH) solution. The cauda epididymis was then cut open to disperse the sperm. The sperm suspension was equilibrated in CO_2 incubator (Heal Force) at 37ºC with 5% of CO_2 for 1 hour prior to sperm parameters evaluation.

Sperm motility: Sperm motility assessment was performed using a Hemocytometer (Hausser Scientific: Improved Neubauer, USA) counting chamber in accordance with the gold standard by World Health Organization (Bailey *et al.*, 2007). The haemocytometer consists of a thick microscope slide with a rectangular indentation that creates two chambers. A glass cover was placed onto the chambers and sperm suspensions, 20 µL were introduced under the cover glass into each chamber. The sperm were allowed to sediment in the grid of the counting chamber for 1 to 2 minutes. The sperm were counted manually for motility trait under the light microscope (Olympus CX21) at 40x magnification.

Sperm viability and morphology: Sperm viability was assessed by the Eosin-nigrosin staining method in order to identify the live and dead sperm. Live sperm do not absorb the Eosin Y stain (unstained sperm) will appear white or fluorescent with an intact cell membrane. Those sperm that show pink or red colours are classified as 'dead' (Kvist and Björndahl, 2002). The Nigrosin stain provides dark background so that sperm will be clearly seen. Sperm suspension, 100 µL was mixed with 100 µL of Eosin-nigrosin stain. The mixture, 15 µL was transferred onto clean glass slide, smeared and left to dry overnight at room temperature, 25ºC. The slides were observed under a light microscope (Olympus CX21FS1) with 40x magnification. A total of 200 lives and dead sperm were counted for each slide for sperm viability (Sharma and Agarwal, 2021). Another 200 sperm were counted for normal and abnormal sperm morphology (WHO, 2010).

Testis histology: Testis was harvested and fixed in formalin prior to histological processes. The fixed testis was rinsed three times for 30 minutes each with 0.1 M phosphate buffered saline (PBS) pH 7.4 (SIGMA: P7059). The testis was then dehydrated in a series of alcohol with ascending concentration for an hour each prior to immersion in an equal part of absolute alcohol and cedar wood oil (SIGMA: 96090) for overnight. The processed tissue was infiltrated with paraffin wax (Leica, Biosystem) to remove excess alcohol from the tissue after the dehydration process. The tissue was next embedded in molten paraffin wax prior to sectioning process.

The tissue in the paraffin block was sectioned at 5µm thickness by using a microtome (Cat no: 08050282, Feather) and mounted on glass slide surface. The sectioned tissue slides were then dried overnight in the oven at 37°C. The dried sectioned tissue was deparaffinised using xylene solution and rehydrated in series of descending alcohol concentration. The slides were then stained with Haematoxylin which dye the nucleus a violet colour. Next, the cytoplasm of the cell was stained pink by the Eosin dye. The stained slides were dehydrated with series of ascending alcohol and cleared again with xylene. Then, the stained slides were mounted with Dibutylphthalate Polystyrene Xylene (DPX), mounting agent to produce a clear binder between the slide and cover slip. The tissue was viewed under 20X and 40X magnification of light microscope (Olympus CX21FS1) for testicular cell count. The spermatogonia, spermatocytes, spermatids, spermatozoa, Sertoli and Leydig cells were counted manually under the light microscope by using NIS-Element Imaging System Software (Nikon) at 20X and 40X magnification.

Statistical Analysis: Statistical analysis of data obtained from sperm parameters and testicular cell count were performed on a microcomputer using Statistical Package for Social Science (SPSS) (Version 20.0) programmed. The various effects on sperm motility, viability, morphology and testicular cell count were analysed using the analysis of variance (ANOVA) and Duncan Multiple Range Test with significant level of $p<0.05$.

RESULTS

Sperm Motility: The analysis of variance for sperm motility indicated that treatment had highly significant effects on this parameter ($p < 0.05$). The Morphine- *P. dactylifera* group showed significantly higher sperm motility ($72.80 \pm 4.48 \times 10^6$ sperm/ml) than the morphine group ($53.52 \pm 3.45 \times 10^6$ sperm/ml, respectively) ($p < 0.05$) (Table 1).

Sperm Viability: The analysis of variance for sperm viability indicated that treatment had highly significant effects on this parameter ($p < 0.05$). Life sperm was found to be significantly higher in Morphine- *P. dactylifera* group (86.65 ± 0.00 %) than the Morphine group (80.76 ± 0.00 %). However, significantly lower dead sperm was observed in the Morphine- *P. dactylifera* group (11.44 ± 0.00 %) than the Morphine group (19.24 ± 0.00 %) ($p < 0.05$) (Table 1).

Table 1. Least square mean analysis of variance for sperm count, motility and viability of rats administered with Morphine and *P. dactylifera*

Treatment	Least Square Means		
	Sperm motility (x 10^6 sperm/mL) (mean ± SE)	Sperm viability (%) (mean ± SE)	
		Live	Dead
Control (n=10)	102.14 ± 1.65^{c}	90.93 ± 0.00^{d}	9.09 ± 0.00^{a}
Morphine (n=10)	53.52 ± 3.45^{a}	80.76 ± 0.00^{a}	19.24 ± 0.00^{d}
P. dactylifera (n=10)	99.24 ± 1.91^{c}	85.50 ± 0.01^{b}	14.50 ± 0.01^{c}
Morphine-*P. dactylifera* (n=10)	72.80 ± 4.48^{b}	86.65 ± 0.00^{c}	11.44 ± 0.00^{b}

^{abcd}superscripts in the same column show significant difference ($p < 0.05$)

Sperm Morphology: The analysis of variance for sperm morphology indicated that treatment had highly significant effects on this parameter ($p < 0.05$). Significantly higher normal (77.35 ± 0.00 %), lower abnormal sperm head (4.63 ± 0.00 %) and tail (17.97 ± 0.00 %) were observed in the Morphine- *P. dactylifera* group in comparison to the Morphine group ($p < 0.05$). *P.dactylifera* group showed the highest in normal sperm (80.33 ± 0.00 %), the lowest of abnormal head (2.67 ± 0.00 %) and abnormal tail (16.95 ± 0.00 %) than the other three groups (Table 2).

Testis Histology: Seminiferous tubules of the control group showed normal arrangement of testicular germ cells. The lumen filled with abundant of spermatozoa and the Sertoli cells were clearly seen in the seminiferous tubules (Figure 1A). Whereas, destruction of the seminiferous tubules in the morphine group was noted with degeneration and disorganization of testicular germ cells. Reduction of spermatozoa in the lumen of seminiferous tubules were noted in comparison to the control group (Figure 1B). Normal arrangement of the testicular germ cells and Sertoli cells were detected in the seminiferous tubules of the *P. dactylifera* extract group. The testicular germ cell layers were thicker as compared to the control group and the lumen was filled with spermatozoa (Figure 1C). The histoarchitecture of seminiferous tubules for the morphine-*P. dactylifera* extract group showed complete stages of testicular germ cells with abundant of spermatozoa found in the lumen than that of the morphine group (Figure 1D).

Table 2. Least square mean from analysis of variance for sperm morphology of rats administered with Morphine and *P. dactylifera.*

Treatment	Least Square Means		
	Sperm morphology (%) (mean ± SE)		
	Normal	Abnormal Head	Abnormal Tail
Control (n=10)	78.21 ± 0.00^{c}	2.96 ± 0.00^{a}	18.77 ± 0.00^{c}
Morphine (n=10)	74.60 ± 0.00^{a}	5.80 ± 0.00^{c}	19.53 ± 0.00^{d}
P. dactylifera (n=10)	80.33 ± 0.00^{d}	2.67 ± 0.00^{a}	16.95 ± 0.00^{a}
Morphine-*P. dactylifera* (n=10)	77.35± 0.00^{b}	4.63 ± 0.00^{b}	17.97 ± 0.00^{b}

^{abcd} superscripts in the same column show significant difference ($p < 0.05$)

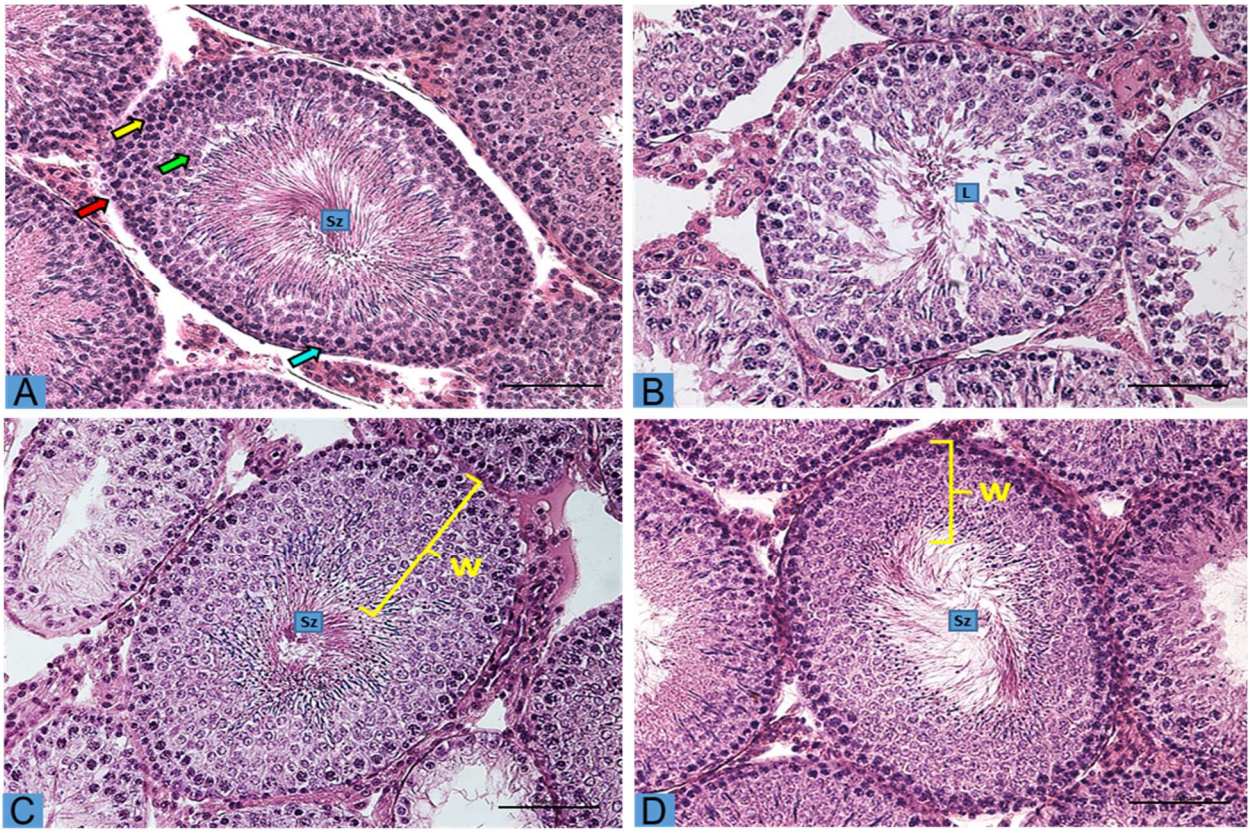

Figure 1: Photomicrograph of transverse section of rat's testis. (A) Control group showed a normal seminiferous tubule has numerous Sertoli cells (Blue arrow), complete stages of testicular germ cells (W), spermatogonium (Red arrow), spermatocytes (Yellow arrow), spermatids (green arrow) and spermatozoa (Sz) in the lumen of the tubule (L). (B) Morphine treated group showed destruction of the seminiferous tubules with less spermatozoa in the lumen. (C) *P. dactylifera* extract group and (D) Morphine-*P. dactylifera* extract group showed complete stages of testicular germ cells.

Testicular Cell Count and Cell Morphometry: The analysis of variance showed that treatments significantly affected the testicular cell count ($p < 0.05$). In general, significantly lower testicular germ cells, Sertoli and Leydig cells count were observed in the morphine group than the other treatment groups. Whereas, the morphine-*P. dactylifera* group found to be significantly higher testicular germ cells (spermatogonia, 63.14 ± 1.90; spermatocyte, 75.14 ± 2.42; spermatid, 226.66 ± 6.16; spermatozoa, 134.82 ± 3.53), Sertoli (10.70 ± 0.42) and Leydig cells (57.54 ± 1.93) count in comparison to the morphine group (Table 3 and 4).

Table 3. Least square means from analyses of variance for testicular germ cell counts of control, morphine, *P. dactylifera* and morphine-*P. dactylifera* extract groups.

Treatment	Mean ± SE			
	Spermatogonia	Spermatocytes	Spermatids	Spermatozoa
Control (n=10)	68.58 ± 1.75^{c}	81.34 ± 2.89^{c}	248.74 ± 10.91^{b}	172.44 ± 7.12^{c}
Morphine (n=10)	43.42 ± 1.39^{a}	51.98 ± 1.51^{a}	143.86 ± 4.30^{a}	72.76 ± 3.32^{a}
P. dactylifera (n=10)	62.62 ± 1.78^{b}	72.82 ± 2.09^{b}	247.02 ± 7.49^{b}	136.04 ± 4.55^{b}
Morphine-*P. dactylifera* (n=10)	63.14 ± 1.90^{b}	75.14 ± 2.42^{bc}	226.66 ± 6.16^{b}	134.82 ± 3.53^{b}

^{abc} different superscripts within the same column show significant differences ($p < 0.05$)

Table 4. Least square means from analyses of variance for sertoli cells and leydig cells of control, morphine, *P. dactylifera* and morphine-*P. dactylifera* extract groups.

Treatment	Mean ± SE	
	Sertoli cells	Leydig cells
Control (n=10)	9.28 ± 0.33 [b]	60.86 ± 2.71 [b]
Morphine (n=10)	6.86 ± 0.32 [a]	41.64 ± 2.59 [a]
P. dactylifera (n=10)	10.22 ± 0.32 [bc]	62.16 ± 2.44 [b]
Morphine-*P. dactylifera* (n=10)	10.70 ± 0.42 [c]	57.54 ± 1.93 [b]

[abc] different superscripts within the same column show significant differences ($p < 0.05$)

DISCUSSION

Opioids have been scientifically proved to be able to accelerate free radical production in the body to an unhealthy state (Ward *et al.*, 2020). Morphine is an opioid which has been widely used as a pain relief medication. On the contrary, morphine has damaging consequences on the male reproductive system. These adverse effects include hypogonadism and severe sexual hormonal imbalances (Karami *et al.*, 2019). Morphine also disrupts regular spermatogenesis and pituitary hypothalamic testicular axis (Roshankhah *et al.*, 2017). Similarly, Roshankhah *et al.* (2020) reported a significant reduction in sperm parameters, total antioxidant capacity (TAC), testosterone level, and germinal layer height (GLH) in the morphine group compared to the control group. Lower sperm count, sperm motility, and rate of spermatogenesis in male rats administered with morphine were also observed by Ahmadnia *et al*. (2016). These findings are similar to the present study that observed a reduction in sperm quality and testicular cell count of morphine group in comparison to the other treatment groups.

In a previous study on the toxicity effects of opioid analgesics, abnormalities observed in the testicular structures of male rats have proven the presence of oxidative damaging effects of opiate free radicals. Oxidation in mitochondrial pores that occurred due to high ROS levels could interrupt the inner and outer mitochondrial membranes (Mishra and Shaha, 2005), causing the opening of the mitochondrial permeability transition pore which subsequently induced the transmembrane potential depolarization (Ly *et al*., 2003). The released of cytochrome C into the cytosol by the loss of transmembrane potential will cause instability of mitochondrial membrane potentials (MMPs). The role of MMPs in maintaining mitochondrial homeostasis and instability of MMPs trigger the apoptotic cascade and loss of oxidative phosphorylation (Gabr and Al-Ghadir, 2012; Zorova *et al*., 2018). These findings could be the reasons for a lower sperm viability due to cell apoptosis in morphine group found in the current study. Furthermore, oxidative damage to the mitochondrial would also affect the production of ATP and impair the energy supply for sperm movement. Damaged mitochondria would probably be one of the reasons for the reduction of sperm motility seen in the morphine group.

In the current study, the morphine treated group showed degeneration and disorganization of spermatogenic cells in testis histoarchitecture of the rats. The spermatozoa were remarkably reduced in the lumina of the seminiferous tubules. The number of testicular cells namely, the spermatogonia, spermatocytes, spermatids and spermatozoa as well as Sertoli and Leydig cells were significantly reduced which indicated the destruction of the testicular histoarchitecture due to morphine consumption. Hakami *et al*. (2022) claimed that opioids exert various effects on the hypothalamic-pituitary function and hypogonadism is their most prevalent endocrine adverse effect. The male hypothalamic-pituitary-gonadal (HPG) axis is–the main controlling system to stimulate spermatogenesis and androgen biosynthesis. Disturbance to HPG axis would lead to reduction in secretion of luteinizing hormone (LH) by the pituitary glands. This reduces the testosterone secretion by the Leydig cells which consequently disrupts the spermatogenesis and leads to reduction in the testicular cell count as observed in the present study. This functional interaction between morphine and testosterone via the effect on HPG axis was in agreement with Fountas *et al*. (2018).

The cellular membrane of the testicular cells contains an abundance of polyunsaturated fatty acid. Lipid peroxidation by opiates can also results in the dysfunction and structural damage to the membrane cells and thus, reduce spermatogenesis (Youssef and Azza, 2016). Takzare *et al.* (2016) also found that morphine could affect all spermatogenesis stages. The population of cells at spermatogonia, spermatocyte, spermatid, and

spermatozoa stages of the spermatogenesis cycle were significantly decreased in those rats that received opioid in comparison to the control group ($p<0.05$). This finding was supported by histological changes in different groups of opioid which affected sperm formation.

The Egyptians have been using date palm as a traditional herbal medicine to improve male and female fertility (Hassan *et al.*, 2012). The aqueous extract of *P. dactylifera* or date palm has been used as a sex enhancer and was shown to cure male infertility which is confirmed by Dillasamola *et al.* (2019). Findings from our present study also supported that *P. dactylifera* is beneficial in male reproductive system. The results showed that the date palm could improve deleterious effects caused by the administration of morphine, in terms of sperm quality, testicular cells and testis histological features. This could be due to the contents of the date palm, which was claimed to be rich in mineral, flavonoid, glycoside and vitamins. Flavonoid is known to be a major class of phytoestrogen that has similar structure and function as estrogen. It has beneficial effects on spermatogenesis as it functions an antioxidant that protects the testis and sperm by absorbing and neutralizing free radicals, quenching singlet or triplet oxygen or decomposing peroxides. The presence of flavonoid in date palm was reported to enhance spermatogenesis, increase seminiferous tubules diameter, and improve sperm morphology (Bahmanpour *et al.*, 2013).

A study by Amirah *et al.*, (2020) reported that *P. dactylifera* could improve the tissue histoarchitecture and function of columnar epithelial cells in the seminal vesicle and prostate gland of morphine-induced rat. The authors further explained that in the morphine group, the seminal vesicle showed the absence of honeycomb-like appearance with flattened columnar cells while in the prostate gland, eosinophilic secretion was absent from glandular lumina in comparison to the control group. These findings provide additional information on the effects of *P. dactylifera* and morphine on male reproductive structures which support the present result that the date palm has the potential to improve the deleterious effect cause by morphine.

Another study by Jahromi *et al*. (2017) in an experimental testicular torsion/detorsion model in rats has also suggested the possible protective effect of the date palm against testicular oxidative damages. Tugba and Yasemine (2018) reported that compounds with high antioxidant capacity, such as phenolic compounds in the date palm extract have positive effects on the prevention and treatment of male infertility. Pre-treatment with the date extract could prevent testicular damage, improved spermatogenesis, and enhanced the testicular structure (Abdu, 2018).

Interestingly, morphine-*P. dactylifera* group of the present study was noted to have complete spermatogenic cells series with spermatozoa filled the lumen based on the histological studies. The histoarchitecture of rat testis was supported by testicular cell counts where the spermatogenic cells, Sertoli and Leydig cells were significantly increased due to *P. dactylifera* supplementation in morphine treated rats. This result was in agreement with El-kott *et al*. (2014) that supplementation of *P. dactylifera* fruit extract showed normal arrangement of seminiferous tubules with abundant of Sertoli cells, different stages of spermatogenic cells layers. Date fruit could also improve sperm cell morphology and reproductive hormonal profiles in cypermethrin-induced male infertility of Wister rats (Ubah *et al.*, 2021). These findings may be attributed to the nutritional values in its potent constituents that make it beneficial for sexual improvement (Mirza *et al*., 2019).

Researchers have related the ameliorative effects of *P. dactylifera* to the action of antioxidant of its nutritional compounds. In accordance with current findings, the natural antioxidant contents in *P. dactylifera* such as vitamins, flavonoids, phenolic and sterols are the key contributors to free radicals' scavengers that prevent morphine-induced oxidative stress in the morphine-*P. dactylifera* group. Further study is warranted to explore the underlying healing mechanism of *P. dacytilfera* at the molecular levels for a better understanding the testicular metabolism which affect human infertility.

Conclusions: *Phoenix dactylifera* has beneficial ameliorative effects which restores normal sperm parameters and histological structure of morphine-induced damages on male reproductive organ. The outcomes are attributed to abundance of potent antioxidants in *P. dactylifera* such as flavonoids and phenolic compounds. These findings have offered a possible solution to the detrimental effects of long-term morphine usage and promotes the use of an inexpensive dietary supplement such as the *P. dactylifera* extract to improve male fertility. This study also contributes to a better understanding on the mechanism that demonstrates the benefit of *P. dactylifera*, which is critical in developing novel treatment modalities for infertility.

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