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ORIGINAL ARTICLE

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Tramadol (opioid) abuse is associated with a dose- and time-dependent poor sperm quality and hyperprolactinaemia in young men

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Summary

Tramadol, one of the most commonly abused drugs in Middle East, impacts spermatogenesis and disturbs reproductive hormones in animal studies. We aimed to investigate tramadol impact on sperm quality and on levels of testosterone, prolactin and gonadotropins, in tramadol abusers (n = 30) to age-matched control (n = 30). Abusers had significantly low percentages of sperm motility, normal forms and vitality compared with control (95% CI −40.7 to −19.3, −13.5 to −9.3 and −31.9 to −9.7 respectively). Hypoandrogenism (95% CI −4.5 to −2.8), hyperprolactinaemia (CI (95%) 4.9 to 9.4) and hypergonadotropinaemia (95% CI 2.9 to 7.2 for FSH and 2.0 to 7.8 for LH) were observed in tramadol abusers vs controls. Smokers (26 of 30), concurrently abusing other drugs (11 of 30) and asymptomatic leucocytospermic (15 of 30) patients subgroups significantly abused tramadol beyond 3 years (p = .02, <.001, = .03 respectively) and in excess >450 mg/day (p = .02, = .01, = .005 respectively). Progressive motility (a + b%) was significantly low in young men <25 years old (p = .03) subgroup. Tramadol abuse is associated with poor sperm quality, hyperprolactinaemia and hypergonadotropic hypogonadism. We recommend semen analysis for tramadol long-intakes, question sperm donors and follow-up studies to prevent and reverse tramadol-induced testicular damage.

KEYWORDS
opioids, prolactin, sperm quality, testicular function, tramadol

1 | INTRODUCTION

Tramadol is one of the most effective prescriptions for treating pain (Ahmad et al., 2017; Awadalla & Salah-Eldin, 2016). It is an opioid analgesic, modifies the pain centrally, through mu receptor agonistic action, and inhibiting serotonin and norepinephrine reuptake in neurons, and locally by increase in nitric oxide (NO) level (Dal, Salman, Salman, Iskit, & Aypar, 2006; Grond & Sablotzki, 2004; Oliva et al., 2002). O-desmethyl-tramadol, an active tramadol metabolite, is 2–4 times as potent as tramadol (Grond & Sablotzki, 2004; Matthiesen, Wohrmann, Coogan, & Uragg, 1998; Tao et al., 2002). Tramadol's half-life (t1/2) is around 6.3 hr; following cessation of intake, it stays 1.44 days for tramadol and 1.7 days for O-desmethyltramadol. Although tramadol is still considered as a nonaddictive drug, reports showed withdraw effects and physical dependence for its long use (Manivannan, Mittal, Goyal, Ansari, & Lohiya, 2009; McDiarmid, Mackler, & Schneider, 2005; Senay et al., 2003).

United Nation reported that middle east and north Africa regions were among highest areas of tramadol/opiate abuse over the world (International-Narcotics-Control-Board, 2013). Tramadol

dependence is more common in youth, with or without other drug addiction (e.g., heroin), smoking, and/or other factors which could induce tramadol accumulation in body systems, decrease its clearance and/or increase its toxic odds (Abou El Fatoh et al., 2014; De Decker et al., 2008; Tjaderborn, Jonsson, Hagg, & Ahlner, 2007). Moreover, men who commonly use tramadol for enhancing sexual dysfunction such as premature ejaculation (PE) may be liable to its potential long-term risks (Abdel-Hamid, Andersson, Waldinger, & Anis, 2016). Reports showed that tramadol induced changes in gonadal hormones and spermatogenesis in laboratory animals (Adams, Sewing, Forman, Meyer, & Cicero, 1993; Ahmed & Kurkar, 2014; Aloisi et al., 2009).

We have designed this study to investigate possible effects of tramadol on testicular function, for the first time, in human and to examine for any possible factor that may alter the association of tramadol with testicular function. We have examined patients' subgroups for any possible cofounder. We want to answer the following questions: is there any relationship between tramadol with testicular function? Does it more affect youth? What are observed factors prevail with tramadol dependence and may alter testicular function? We have examined semen concentration, sperm motility, vitality and sperm morphology as well as serum levels of gonadotropins, prolactin and total testosterone in men presented for drug addiction clinic in Menoufia University Hospital.

2 | MATERIALS AND METHODS

We have started our study following its approval from the committee of Human Rights in Research at Menoufia University Hospital, Shebin Elkom, Egypt, on 12 October 2014. Enrolling patients in this study, illustrated in suppl. Figure 1, had begun after signing an informed consent. Initially, 55 tramadol abusers who were pursuing medical help at drug addiction clinic, Forensic and Clinical Toxicology, Menoufia University Hospitals from November 2014 and October 2015 were enrolled. Healthy volunteers and patients were clinically evaluated in the Andrology clinic (Figure S1). Inclusion criteria were mainly daily tramadol use at the last 3 months, and ability to produce semen sample and give 10 ml blood with the absence of exclusion factor. Exclusion conditions were presence of any local and/or systemic disease that may affect testicular functions such as fever, chronic disease (liver, kidney …etc.), obesity (BMI), diabetes, under hormonal treatment or androgen therapy, testicular pain, varicocele, testicular mass, and/or inability to provide semen or blood samples. Applying inclusion/exclusion criteria shortlisted our study enrolled subjects to thirty abusers who were finally examined and compared with thirty matched healthy men to serve as study controls (Figure S1).

Subgroup classifications and analysis were performed to examine for any possible confounders or any factor that may affect the association of tramadol dependence with testicular function as shown in Figure S1:

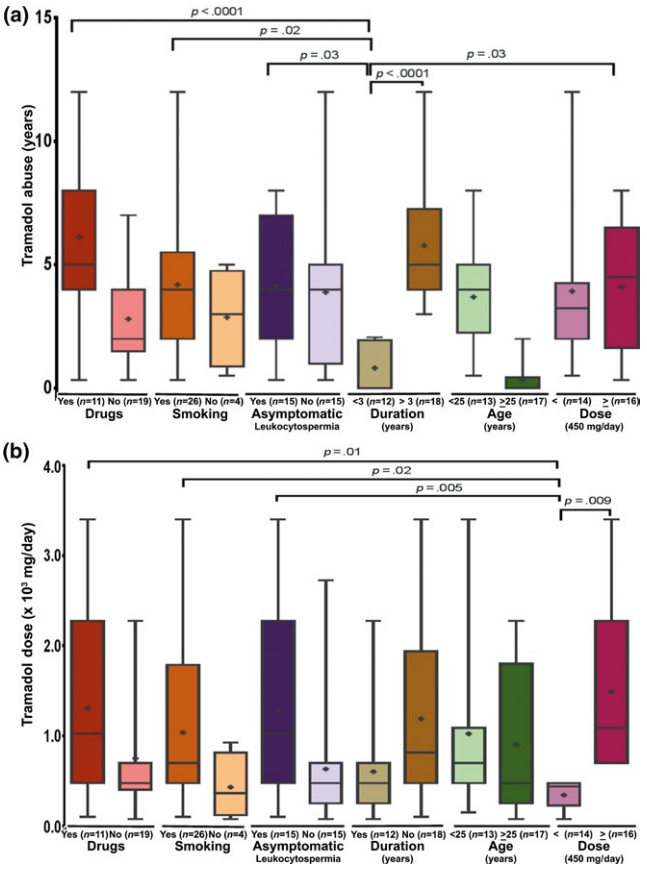

FIGURE 1 Tramadol abuse factors; (a) duration (y) in overall patients and their subgroups. For comparison purposes, short duration group was used as a control group to compare with other patients subgroups regarding their abuse duration. Statistical examination used short duration subgroup (<3 years) and for (b). daily dose (g), low daily dose (450 mg/day, pharmacological range) patients' subgroup was used to compare with other patients regarding their daily tramadol dose. Two-way analysis of variants was used for multiple comparisons of patients' subgroups followed by Holm–Sidak correction; *p*-values between each two subgroups were provided for comparison between each two subgroups. Results got represented as box and whiskers plots used min and max range, plus sign refers to mean

a) Concurrent addiction of any agent (e.g., heroin, opioid, hashish ("hash"), and alcohol) into the only tramadol with no additional (Tram-ND), or presence of additional addiction agents (Tram + D).
b) Smoking status, non-smoker or smokers, included mild–moderate and heavy smoker based on their daily smoking habit (Boulos et al., 2009) into Tram + S indicates smoking plus tramadol abuse or TramNS if only tramadol abuse with no smoking.
c) Presence of asymptomatic leucocytospermia although tramadol abuser (n = 11) were excluded if fever, infection or pain was present, some showed leucocytospermia >1 million/ml (World Health Organization, 2010). Tramadol abusers were subdivided into Tram + L if leucocytospermia >1 or Tram-NL if leucocytospermia ≤1.
d) Duration of tramadol abuse; subgrouped into <3 or ≥3 years. Three years was chosen as an observed cut-off of semen deficit with tramadol use in normozoospermic patients.

e) Age; abusers were subgrouped based upon their age into <or ≥25 years as per literature. Many drug abuses were among younger youth <25 years.
f) Total dose of tramadol used daily for every patient, into, beyond recommended a pharmacological dose or not, <450 mg per day or above 450 mg daily.

All participants in this study were subjected to a thorough medical history with special emphasis on medication use or drug abuse. Medical examination was carried out to fulfil the inclusion and exclusion conditions determined for this study. Daily tramadol dose was calculated as follows; total tramadol per day = Tramadol tablet number x tablet concentration (225 mg). Few patients have used 50- or 100-mg tablet instead of 225-mg tablet. Duration of tramadol abuse was given in years; the minimum duration of tramadol use in enrolled men was 3 months, which equals 0. 25 years.

2.1 | Semen analysis

Each semen sample was collected and analysed in a clinical pathology laboratory, University Hospital, according to WHO 2010 Guidelines (World Health Organization, 2010). All semen specimens were tested for conventional sperm parameters such as concentration (million/ml), total motile spermatozoa (million/ml), progressive motility (grade a + b, %) and normal morphology forms (%) as per WHO criteria. Sperm vitality (%) was determined using eosin-nigrosin staining, and leucocytic concentrations were confirmed by Endtz testing to examine for leucocytospermia (million/ml) (Desai, Mahfouz, Sharma, Gupta, & Agarwal, 2010; Mahfouz, Sharma, et al., 2010).

2.2 | Hormonal levels of FSH, LH, testosterone and prolactin

Five millilitres of blood was withdrawn from every man into a red-capped tube under complete aseptic conditions. After 15 min, all tubes were centrifuged for 10 min at a speed of 500 *g* for serum separation. Harvested serum was aliquoted into four small tubes, each 100 μl and kept at −20°C until runtime. Each hormone was examined as a batch for all samples of study groups. Enzyme immunoassay test kits from Chemux BioScience Inc., CA, were used to examine serum for follicle stimulating hormone (FSH) in mIU/ml (Cat # 10001), luteinising hormone (LH) in mIU/ml (Cat # 10004), prolactin (Cat # 10006) in mIU/ml and total testosterone in ng/ml (Cat # 10007), according to manufacturer's recommendation.

2.3 | Statistical analysis

Sample size was estimated, based upon a pilot data, as 30 per group, using sperm motility (%) as the primary variable, assuming to detect a difference of 10% at an alpha threshold of 5%. Data were examined for their distribution by Kolmogorov–Smirnov test; data presented with either as mean ± *SD* as seen in Table 1 or median and inter-quartile (IQ) ranges as shown in figures. Comparisons of healthy control vs patients' (sub)groups were examined using unpaired *t* test, 95% confidence intervals (95% CIs) at 5% alpha,

TABLE 1 Comparison of sperm parameters and serum hormones in overall tramadol abusers vs healthy control

Parameters	Patients (*n* = 30)	Control (*n* = 30)	CI (95%)
Semen analysis			
Volume (ml): X ± *SD*	2.1 ± 0.9	2.2 ± 0.5	−0.48–0.28
Sperm conc. (mil/ml): X ± *SD*	46.9 ± 26.9	49.6 ± 23.7	−15.8–10.4
Progressive motility (a + b%): X ± *SD*	46.2 ± 28.6	76.2 ± 6.8	**−40.7 to −19.3**
Sperm vitality (%): X ± *SD*	51.2 ± 29.1	72.0 ± 8.9	**−31.9 to −9.7**
Normal forms (WHO, %): X ± *SD*	5.1 ± 2.4	16.5 ± 5.1	**−13.5 to −9.3**
A. leucocytic count (Mil./ml): X ± *SD*	1.1 ± 0.69	0.55 ± 0.23	**0.28–0.82**
Serum hormones			
Total T. (ng/ml): X ± *SD*	2.09 ± 0.9	5.8 ± 2.1	**−4.5 to −2.8**
PRL. (mIU/ml): X ± *SD*	13.9 ± 5.5	6.7 ± 2.5	**4.9–9.4**
FSH (mIU/ml): X ± *SD*	16.6 ± 4.3	11.5 ± 3.9	**2.9–7.2**
LH (mIU/ml): X ± *SD*	15.2 ± 6.6	10.3 ± 4.2	**2.0–7.8**

Significance is shown in bold 95% CI range. An increased incidence of asthenozoospermia, necrozoospermia, low percentage of normal sperm WHO morphology and increased incidence of asymptomatic leucocytospermia in patients. Low total testosterone with high prolactin and FSH and LH was seen in tramadol abuser patients compared with control group.
A, asymptomatic; CI, 95% confidence interval; FSH, follicle stimulating hormones; LH, luteinising hormones; PRL, prolactin; *SD*, standard deviation; T, testosterone; X, mean; bold 95% CI is significant using unpaired *t* test (https://www.graphpad.com/quickcalcs/ttest1.cfm).

were used, preferable to *p*-values, to show significant differences in Table 1, verified on https://www.graphpad.com/quickcalcs/ttest1/?Format=SD. Two-way analysis of variance was performed to compare patients' subgroups and healthy controls, followed by Holm–Sidak correction for multiplicity adjusted *p*-values to examine any significant differences between patients' subgroup(s) vs. healthy control. $p < .05$ was considered statistically significant. All statistics were performed using GraphPad Prism version 7.03 for Windows, GraphPad Software, San Diego, California, USA, www.graphpad.com. Principle component analysis, models the variation in a set of variables in terms of a smaller number of independent linear combinations, was performed on multiple correlated variables for all patients and control (sub)groups, Figure S2, using JMP version 13 (SAS Institute Inc, Cary, NC, http://www.jmp.com), analysis R script is attached as supplemental word file.

3 | RESULTS

3.1 | Demographic findings

Logistics of this study were detailed in Figure S1 with colour coding for control and patients (sub) groups. Age of tramadol abusers ($n = 30$) ranged from 18 to 35 years, with mean ± *SD* equals 25.4 ± 4.6 years, which was compared with control age ranged from 18 to 37 with mean ± *SD* of 27.5 ± 5.6 ($n = 30$, $p = .12$, Table S1). Patients group could be subclassified into smokers ($n = 26$) subgroup which had mean age ± *SD* of 25.6 ± 4.7 while non-smoker patients ($n = 4$) had 24.5 ± 3.9. Smokers of control group ($n = 13$) showed mean age ± *SD* of 25.1 ± 4.9 and 29.2 ± 5.4 for non-smokers ($n = 17$).

Body mass index (BMI) of patients was compared with control groups; BMI is not significant ($p = .9$); mean BMI ± *SD* patient had of 24.2 ± 4.6 vs 24.2 ± 3.9 for control group. Smoking was so significant in overall patients group (26/30, 87%) vs. control group (13/30, 43%), $p = .0012$ using chi-square with Yates' correction. Smoking showed an odds ratio of 8.5 with a 95% confidence interval of 2.37–30.48 (Table S1).

Tramadol abuse was stated to premature ejaculation in one of 30 patients (3%), to psychic/social problems in 11 of 30 patients (37%), to nonmedical use in 14 of 30 patients (47%) and to psychiatric/mode disorders in seven of 30 patients (23%). Moreover, 11 of 30 patients (37%) have abused tramadol only, while 19 of 30 (63%) patients had other drug addiction in addition to tramadol abuse. Additional addicted substances were reported as follow: hashish ($n = 6$); morphine ($n = 1$); astral ($n = 1$); alcohol ($n = 1$); heroin ($n = 2$); and two patients have abused two or more drugs/substances.

3.2 | Seminogram and hormonal levels

Patients had comparable semen volume and sperm concentration to control group. However, patients had low percentages of progressive sperm motility (a + b), sperm vitality and normal sperm forms (Table 1) compared to control group. Patients also had higher incidence of asymptomatic leucocytospermia compared with control ($p = .002$, Table 1). Same significant differences remained as shown in Table 2 when patients and control subjects got subclassified into smokers and non-smokers. Sperm concentration was comparable between smoker and non-smoker in both patient and control groups. There were significant differences in sperm motility, vitality and percentage of normal forms (Table 2).

TABLE 2 Sperm quality and serum hormonal levels in smokers stratified in both tramadol abusers and healthy control men groups

	Smokers		Non-smokers	
Parameters	**Patients ($n = 26$)**	**Control ($n = 13$)**	**Patients ($n = 4$)**	**Control ($n = 17$)**
Sperm quality				
Sperm Conc. (mil/ml): X ± *SD*	44.7 ± 24.8	51.3 ± 22.6	61.3 ± 31.7	49.0 ± 22.9
Progressive motility (a + b%):X ± *SD*	**39.5 ± 18.2**	75.2 ± 5.9	**43.7 ± 19.4**	87.2 ± 5.6
Sperm vitality (%): X ± *SD*	**47.0 ± 29.0**	79.0 ± 11.0	75.3 ± 10.0	93.0 ± 6.8
Normal forms (WHO, %): X ± *SD*	**2.5 ± 1.6**	15.1 ± 2.0	**3.7 ± 1.9**	19.7 ± 4.7
Serum hormones				
Total T. (ng/ml): X ± *SD*	**2.0 ± 0.9**	5.8 ± 2.0	**2.6 ± 0.5**	5.8 ± 2.2
PRL. (mIU/ml): X ± *SD*	**14.0 ± 5.6**	7.5 ± 2.9	**12.7 ± 3.8**	6.2 ± 2.1
FSH (mIU/ml): X ± *SD*	**16.3 ± 0.5**	9.5 ± 4.1	**18.5 ± 2.3**	12.8 ± 3.0
LH (mIU/ml): X ± *SD*	**15.5 ± 6.9**	10.1 ± 3.9	13.1 ± 3.5	10.3 ± 4.4

Tramadol patients, in bold, showed significant differences to their corresponding control groups, two-tailed $p < .05$ using unpaired *t* test. Progressive sperm motility, normal morphology, serum prolactin, testosterone and FSH were affected in patients regardless their cigarette smoking status. Sperm vitality and serum LH were only affected in smoker patients.

Significant low total testosterone was observed in patients compared with control (95% CI −4.5 to −2.8). High prolactin level was significantly in patients vs control group (95% CI 4.9–9.4, Table 1). Patients had a significant high FSH and LH levels compared with control group (95% CI 2.9–7.2 and 2.0–7.8, respectively, Table 1). These findings were similarly observed as shown in Table 2 when patients and control subjects got stratified into smokers and non-smokers. Levels of serum total testosterone, prolactin and FSH were significantly different in tramadol abusers vs their control group, regardless their smoking status (Table 2). Serum LH level was comparable between non-smokers patients vs non-smoker controls (Table 2).

Asthenozoospermia was observed in only 35.7% (five of 14) of men who had abused ≤2 tablets (2 × 225 mg) daily, while it occurred in 69% (11 of 16) of men who abused more than two tablets daily, with an odds ratio (OR) of 3.9 and 7.7 for asthenozoospermia and necrozoospermia respectively. For example, pt # 23, a 30 years old, abused daily 50 mg tramadol for 2 years, his seminogram showed 56 million motile spermatozoa with a 70% progressive motility. On the other hand, pt # 42, who was 24 years old and abused 900 mg tramadol daily for 5 years, resulted in only 1.8 million motile spermatozoa with 8% progressive motility in his seminogram. In this examples, we realised that it is basically dose-/time-dependent testicular damage which may end into primary testicular failure. Both patients # 23 and # 42 were non-smokers and never abused other drug/substance concurrently.

Men who abused >3 × 225 mg tramadol daily showed significantly low sperm concentrations (million/ml) mean ± *SD* of 36.7 ± 25.3, $n = 16$ compared with 65.9 ± 23.5, $n = 14$ in men who abused ≤2 × 225 mg tramadol daily ($p = .02$, unpaired t test). Oligozoospermia OR, in men abused excess tramadol, was 2.25 compared with all abusers. Moreover, men, who abused tramadol for ≥3 years, showed a significant low progressive sperm motility (%), median (25, 75 percentiles) of 8% (4.25%, 41%), $n = 18$, vs 45% (13.75%, 70%), $n = 12$, in men who abused tramadol for shorter time ($p = .03$, Kruskal–Wallis Test).

3.3 | Tramadol effect and subgroup analysis

We have examined several patients' subgroups (Figure S1) to search for any dependence as explained above in methodology. As described above in statistical analysis, we have examined several patients' subgroup using MAONVA followed by Sidak correction.

3.3.1 | Concurrent substance abuse

Patients were subclassified into two subgroups according to concurrent additional drug/substance abuse; yes ($n = 11$) or tramadol only ($n = 19$) (Figure S1). Patients with concurrent additional drug/substance abuse had abused tramadol for longer time ($p < .001$, Figure 1a) and in excess ($p = .01$, Figure 1b). Significant decline of progressive motility (a + b%) was observed in them ($p = .02$, Figure 2a), as well as of vitality ($p = .04$, Figure 2b) vs. healthy control group. An increase of serum LH levels ($p = .03$, Figure 3a) was observed in tramadol only subgroup without any additional addiction. Concurrent additional drug abuse had no effect on serum LH levels (Figure 3a), FSH levels (Figure 3b), prolactin levels (Figure 4a) and total testosterone (Figure 4b) when compared to the absence of leucocytospermia subgroup.

3.3.2 | Smoking status

Tramadol abuser patients were stratified into two subgroups; smokers ($n = 26$) or non-smokers ($n = 4$) (Figure S1). Smokers had abused tramadol for longer time ($p = .02$, Figure 1a) and in excess ($p = .02$, Figure 1b). Significant decline of progressive motility (a + b%) was observed in smokers ($p = .002$, Figure 2a) and vitality ($p = .007$, Figure 2b) vs. healthy control group. An increase in serum LH levels ($p = .03$, Figure 3a), FSH levels ($p = .001$, Figure 3b) and prolactin levels ($p < .001$, Figure 4a) were noted in smokers subgroups compared with healthy control group. A decline of total testosterone levels was noted in smokers subgroups ($p < .001$, Figure 4b) compared with healthy men.

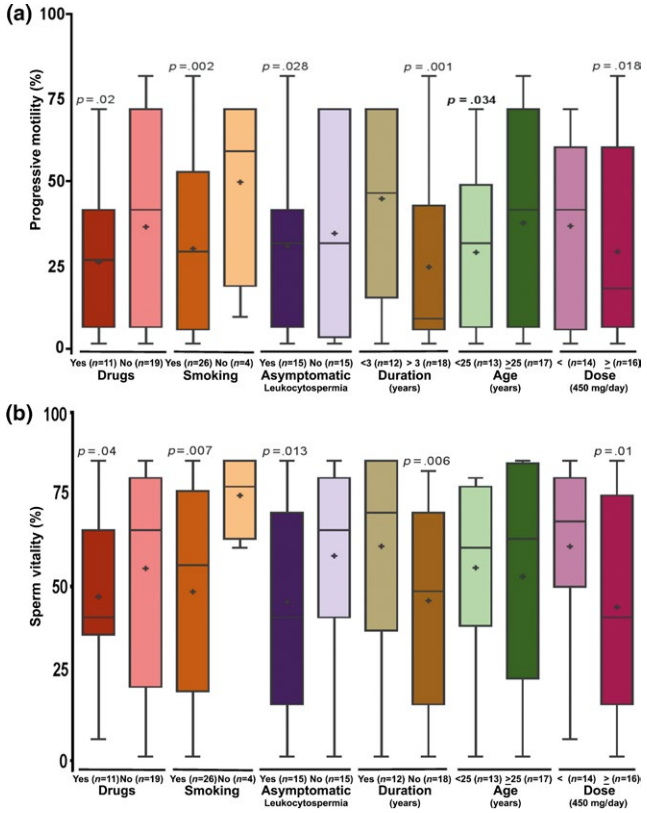

FIGURE 2 Sperm progressive motility and vitality. (a) Sperm progressive motility (a + b), interestingly, young abusers (<25 Y) had significantly low progressive motility. (b) Sperm vitality in patients' subgroups. Significant differences for each subgroup vs healthy control group were shown as *p*-value above each patient's subgroup. Patients' subgroups got compared to the reference healthy control groups for sperm progressive motility and sperm vitality. Box and whiskers plots with minimum and maximum range, plus sign refers to mean

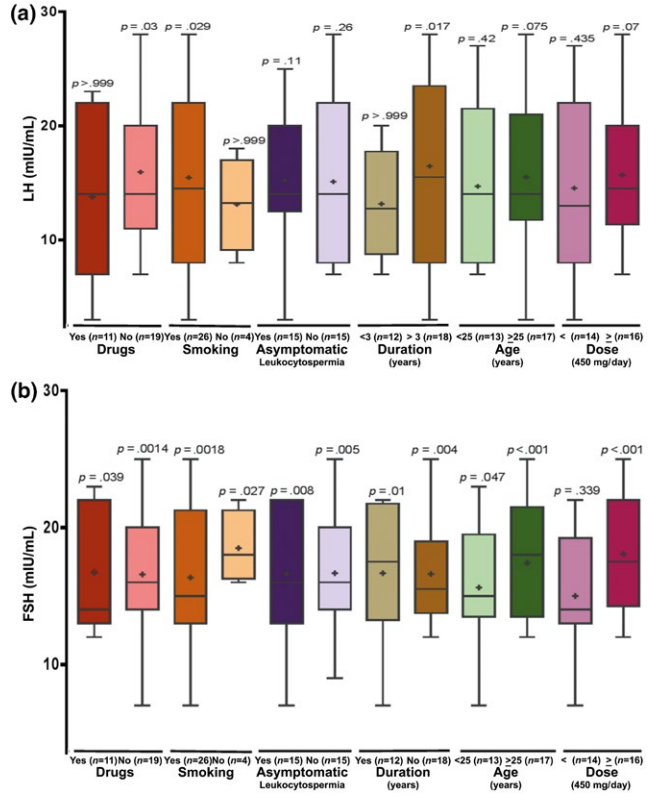

FIGURE 3 Serum levels of LH (a) and (b) FSH, in patients' subgroups compared to healthy control (not shown) using two-way ANOVA for multiple comparisons followed by Sidak correction, box and Whiskers plots with min and max, plus sign refers to mean. Increased LH in patients who consumed no other drugs with tramadol, smokers and abused tramadol for >3 years. FSH elevation was shown in overall patients and subgroups except men who consumed <450 mg tramadol daily

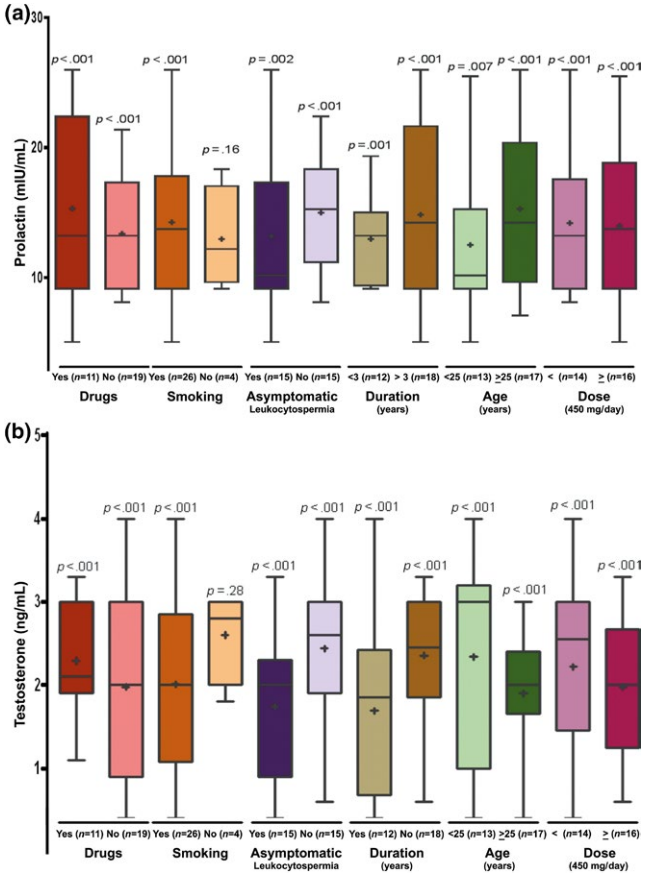

FIGURE 4 Serum prolactin (a) and total testosterone (b) levels to compare multiple patients' subgroups vs healthy group followed by Sidak test correction, represented as box and whiskers plots with min and max for all patients' subgroups, plus sign refers to mean. Except for non-smoker patients subgroup (n = 4), striking upsurge of prolactin and significant decline of total testosterone were observed in overall patients and their subgroups

3.3.3 | Asymptomatic leucocytospermia

Tramadol abuser patients got classified into two subgroups according to the presence of asymptomatic leucocytospermia into; yes (n = 15) or no (n = 15) (Figure S1). Patients with asymptomatic leucocytospermia had abused tramadol for longer time (p = .03, Figure 1a) and in excess (p = .005, Figure 1b). Significant decline of progressive motility (a + b%) was observed in smokers (p = .028, Figure 2a) and vitality (p = .013, Figure 2b) vs. healthy control group. The presence of asymptomatic leucocytospermia had no effect on serum LH levels (Figure 3a), FSH levels (Figure 3b), prolactin levels (Figure 4a) and total testosterone (Figure 4b) when compared to absence of leucocytospermia (no) subgroup.

3.3.4 | Patients' age

We have classified tramadol abuser patients into two subgroups; below or above all patients' mean of age (Figure S1). There were no significant differences in tramadol abuse duration (Figure 1a) or in total daily dose (Figure 1b). Significant decline of progressive motility (a + b%) was observed in patients below 25 years subgroup (n = 13, p = .034, Figure 2a), although sperm vitality was comparable in age subgroups (Figure 2b) vs. healthy control group. A comparable level of serum LH was noted in both patients' subgroups compared with healthy control group (Figure 3a). There were significant increases in FSH levels in both patients below 25 (p = .047) and above 25 (p < .001) years compared with control group (Figure 3b). Moreover, there was an elevation of serum prolactin levels in patients below (p = .007) or above 25 (p < .001) years subgroups compared with healthy control group (Figure 4a). Lastly, both age subgroups (p < .001 for both) confirmed hypogonadism as their testosterone levels were lower compared with healthy men (Figure 4b).

3.3.5 | Duration and daily dose of tramadol

Duration of tramadol abuse (n = 30), Figure 2a, median (interquartiles) was 4 (2, 5) years. Observation-based sperm motility

changed within 3 years of tramadol use within therapeutic range, patients got subgrouped into ≤3 and >3 years subgroups. Interestingly, patients who concurrently abused substance/drugs (e.g., heroin, alcohol, hashish.., Table S1) were smokers, had asymptomatic leucocytospermia and abused excess daily tramadol ($p < .001$, =.02, $p = .03$ and $p = .03$, respectively, Figure 2a). Longer tramadol abuse >3 years decreased percentages of sperm motility (a + b, $p = .001$, Figure 2a) and vitality ($p = .006$ Figure 2b). There was an increase in serum LH ($p = .017$, Figure 3a) compared with control group. Duration of tramadol abuse did not affect changes in serum levels of FSH (Figure 3b), prolactin and total testosterone (Figure 4a–b).

All tramadol abusers received an average of 3 × 225 mg tablets per day (Figure 2b), with the median (inter-quartile) being 675 mg (431.3 mg, 1281 mg) per day. Moreover, overall patients consumed surplus of daily tramadol ($p = .04$, maximum recommended dose for pain is 450 mg). Abusing high daily tramadol dose >450 mg decreased percentages of sperm motility (a + b, $p = .018$, Figure 2a) and vitality ($p = .01$ Figure 2b). There was an increase in serum FSH ($p < .001$, Figure 3b) compared with control group. Daily dose of abused tramadol did not affect changes in serum levels of LH (Figure 3a), prolactin and total testosterone (Figure 4a–b).

3.4 | Multivariable analysis using principle component analysis (PCA)

Using a PCA model, we have examined the arrangement of points across many correlated variables; PCA was used to show the most prominent directions of these input variables in patients and control (Figure S2) which may direct tramadol effect on patients. While 1st PC is the linear combination of the standardised original variables that have the greatest possible variance, each subsequent PC is the linear combination of the variables that have the greatest possible variance and is uncorrelated with all previously defined components. The PC1 accounted for almost 40% of the variation in the data. Together, the first 2-PCs caused 56.9% variation in the data. Tramadol total dose and duration as well as smoking, additional addicts, leukocytospermia, have large positive loadings on component 1, so PC1 focuses on patients long-term tramadol abuse risk factors such as, duration/dose, nicotine smoking, additional drug addictions and asymptomatic leucocytospermia. Prolactin was strongly associated with duration and total dose of tramadol abuse (Figure S2). Patient's age and percentage of normal forms showed negative loadings on PC2, so PC2 describes impact of tramadol on spermatogenic status. Interestingly, sperm vitality and progressive motility have positive loading on PC2 and negative loading on PC1 which focused on tramadol effects.

4 | DISCUSSION

Opioid-related dependence is a substantial contributor to the global disease burden. Sub-Saharan Africa and Middle East are among the most affected regions (Degenhardt et al., 2014; Progler, 2010). In 2010, 15.5 million opioid-dependent people were reported, with higher prevalence in men than women and peaked at ages of 25–29 years. The International Narcotics Control Board (INCB) reported that about 120 million tablets containing tramadol in 2011 and about 320 million tablets in the 1st quarter of 2012 were seized in Egypt (International-Narcotics-Control-Board, 2013). Unfortunately, the WHO Expert Committee on Drug Dependence 36th ascertained that a critical review of tramadol is not justified at this time based on the available evidence(s) regarding tramadol dependence, abuse and risks to public health (WECoD Dependence, 2014).

In this study, tramadol long-term abusers had low-quality semen profile in terms of low vitality, decreased progressive motility (a + b) and associated with high incidence of leucocytospermia as well as of abnormal sperm defects. Disturbed hormonal profiles were noticed in tramadol abusers patients, as seen in having high FSH, LH and prolactin with low androgen levels. Smoking and other drug addiction may also potentiate deterioration of sperm motility and vitality. Our findings agree with previous reports of tramadol association of hyperprolactinaemia and hypogonadotropic hypogonadism (Abdellatief, Elgamal, & Mohamed, 2015; Ahmed & Kurkar, 2014; Fitzgerald & Dinan, 2008; Osadolor & Omo-Erhabor, 2016). The increased prolactin level could result from central vs peripheral tramadol effect on monoamine reuptake and its differential action on hypothalamic–pituitary axis, where it increases hypothalamic factors that increase prolactin contrast to little tramadol direct stimulation of lactotrophic cells in the pituitary (Nna, Akpan, & Osim, 2016; Osadolor & Omo-Erhabor, 2016). Low androgen and increase hypothalamic release could explain hypergonadotropinaemia. Moreover, level of serum FSH and LH, as part of opioid effect on gonadotropin-releasing hormone (GnRH), get affected by hyperprolactinaemia (Bliesener et al., 2005). Furthermore, Pimpinelli et al., (2006) stated that testosterone synthesis could be directly inhibited by increased prolactin serum levels (Pimpinelli et al., 2006).

More evidences of direct tramadol-induced testicular damage originated from animal studies which support our findings on deteriorated sperm quality parameters such as count, motility and vitality ending into low fertility potential (Abdellatief et al., 2015; Ahmed & Kurkar, 2014; Azari et al., 2014; Ceccarelli, De Padova, Fiorenzani, Massafra, & Aloisi, 2006; Nna & Osim, 2017; Osadolor & Omo-Erhabor, 2016). Severe sperm deterioration may occur from the association of leucocytospermia, smoking, additional drugs abuse with tramadol. Several reports supported that sperm DNA integrity is compromised with any condition, drugs or injurious exposure to testicular tissue affecting spermatogenesis (Abedi, Nabi, Mangoli, & Talebi, 2017; Aziz, Sharma, Mahfouz, Jha, & Agarwal, 2011; Cui, Jing, Wu, Wang, & Li, 2016; Desai et al., 2010; Lackner, Agarwal, Mahfouz, du Plessis, & Schatzl, 2010; Mahfouz, Sharma, et al., 2010; Nna et al., 2016; Talebi, Sarcheshmeh, Khalili, & Tabibnejad, 2011).

Sperm damage may result from oxidative stress by direct tramadol effect on testicular tissues as previous reports (Aziz et al., 2011; Desai et al., 2010; Lackner et al., 2010; Lakpour et al., 2012; Mahfouz, du Plessis, et al., 2010) confirmed it on animal studies in

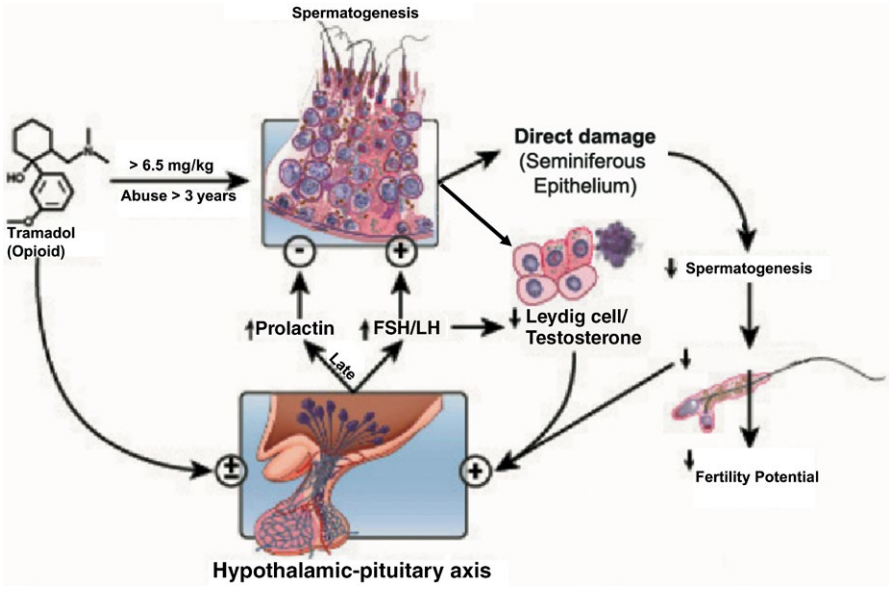

FIGURE 5 Abstracting graph summarises our findings as well as previous reports in the literature of possible effects of tramadol abuse on seminiferous tubular epithelium and Leydig cells resulting in disturbed spermatogenesis and hypogonadism, that is testicular failure. It shows also how tramadol may induce disconcerting hypothalamic–pituitary–testicular axis in the form of hyperprolactinaemia, reflex hypergonadotropism. It has been drawn to show proposed mechanistic effects of tramadol on sperm quality and on reproductive hormones levels. Asthenozoospermia was used as one of sperm quality parameters which got deteriorated earlier. Early testicular changes may result from oxidative stress-induced damage to spermatogenesis (low sperm quality) and also to Leydig cells (low testosterone). Feedback signalling works mainly here as well as additional direct central effect on hypothalamic–pituitary levels resulting in increase in FSH, LH and prolactin hormones. Late changes will end into testicular failure, hypergonadotrophic hypogonadism and hyperprolactinaemia

agreement with this study's findings. Damage of Leydig cells may cause hypoandrogenaemia, from low testosterone production with feedback increase in LH. Damage to spermatogenesis precursors will compromise sperm quality in terms of increase in abnormal forms, low sperm motility and low sperm vitality (Ahmed & Kurkar, 2014). Our findings showed a negative correlation of sperm count, motility and vitality with tramadol abuse dose and duration. From the literature and current study findings, Figure 5 illustrates possible effects reported on tramadol long-term use on reproductive biology.

Testicular tissues showed malformed (Abdellatief et al., 2015) and reduced number (Ghoneim, Khalaf, Elsamanoudy, & Helaly, 2014) of Leydig cells after chronic administration of tramadol. Furthermore, Dal et al., 2006 described a suppressing influence of nitric oxide (NO) on testosterone secretion by Leydig cells in rats (Dal et al., 2006). Evaluation of testicular tissue after 6 weeks of tramadol administration led to straight testicular atrophy resulting in low sperm density in the epididymal lumen, depressed motility and sperm structural aberrations (Azari et al., 2014).

Abdellatief et al., 2015 reported extensive degenerative changes in spermatogenic and Sertoli cells in testicular tissues after intraperitoneal injection of 40 mg/kg tramadol hydrochloride for 30 days (Abdellatief et al., 2015). Sertoli cells showed apical sloughing and shedding with disorganisation of germ cells (Manivannan et al., 2009; Rashed, Mohamed, & El-Alfy, 2010). Our results revealed that tramadol negatively impact male reproductive health on both semen and hormonal profiles. It is greatly affected by abuse duration and tramadol dose as well as any additional effect of other factors such as smoking, other addiction (Marijuana, heroin, hashish….etc.) and presence of leukocytospermia as shown in Table 1 and Figure 1. These factors work mainly through dose and time keystone parameters of tramadol abuse as well as potentiation of direct testicular tissue damage and not through hormonal disturbance as a unique indirect effect of tramadol abuse (Figure 5). Our findings explain and support previous reports (Aloisi et al., 2009; Cui et al., 2016; Dong et al., 2016; Fitzgerald & Dinan, 2008; Lackner et al., 2010; el-Shoura et al., 1995; Yang et al., 2017). Moreover, in human study, Agirregoitia et al., 2006 recorded low sperm motility in heroin users (Agirregoitia et al., 2006). Interestingly, long-term administration of tramadol on animals models revealed that its adverse effects on testes were reversible (Azari et al., 2014; Ghoneim et al., 2014). Cessation of tramadol abuse could be related with testicular function restoration and is still unclear as it requires a follow-up study for confirmation of seminal these patients in the future.

Our paper has few limitations such as the ability to prospectively follow-up abusers for detecting early sperm quality decline, direct measurement of tramadol in blood to accurately correlate its level with risk hazard on a well-timed pattern and lastly controlling to other possible confounders such as social lifestyle, job-related hazards. It would be better to examine changes of semen and serum hormones in these abusers following their recovery in toxicology clinic. Authors recommend future large-scale studies to address all these limitations with use of molecular semen biomarkers (Mahfouz, Ramos, & Kremer, 2012).

In conclusion, this is the first human report; tramadol abuse in young men may result in deleterious effects on testicular function in terms of poor sperm quality, and low testosterone levels in a dose- and time-dependent mode that impulse increase in gonadotropins. Indirectly hyperprolactinaemia may add to the tramadol-induced hypergonadotropic hypogonadism. Other potential factors are common in tramadol abusers such as smoking, concurrent abusing other drugs/substance and the presence of asymptomatic leucocytospermia, may additionally contribute to tramadol-induced testicular failure. We recommend follow-up for men planning to take tramadol beyond 3 months especially younger than 25 years old. We strongly recommend asking about tramadol (opioid) use to all sperm donors, especially young men in andrology laboratory. Large-scale studies may be recommended for a follow-up study on tramadol-induced testicular impairment and its prevention options.

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