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Original Research Article

Fmrfamide Mitigated MDMA (3, 4- Methylenedioxymethamphetamine)/Tramadol-Induced Testicular Toxicity in Wistar Rat

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ABSTRACT

MDMA (3,4-methylenedioxymethamphetamine) and tramadol are central nervous system stimulants known for inducing emotional communion and socializing effects. FMRFamide, an RFamide peptide isolated from mollusks, plays diverse roles in physiological processes. This study aimed to evaluate FMRFamide's impact on testicular toxicity induced by MDMA/tramadol in Wistar rats. Thirty male Wistar rats were divided into six groups (n=5). Group A served as the control, Group B received 2 mg/kg FMRFamide for two consecutive days. Groups C, D, and E received MDMA/tramadol (20 mg/kg) for ten days, while Group F received the same dosage along with FMRFamide. Sperm analysis, biochemical, and histological assessments were conducted. The results revealed significant body weight loss, decreased sperm count and motility, increased abnormal sperm formation, and reduced serum LH, FSH, and Testosterone levels. Inducible Nitric Oxide Synthase (iNOS) expression was intensified in Groups A, B, D, and F. Notably, FMRFamide administration attenuated the adverse effects of MDMA/tramadol-induced testicular toxicity, suggesting a potential therapeutic role in safeguarding male reproductive health. These findings highlight the importance of further exploring FMRFamide's protective mechanisms and its potential application in mitigating drug-induced reproductive toxicity.

Keywords: MDMA, Tramadol, FMRFamide, Testicular Toxicity, sperm cells, reproductive health.

Introduction

Drug abuse is known to be an excessive intake of any substance such as psychoactive drugs, pain medications and other illegal drugs which can lead to difficulties with work, social relationship emotional harm and other long-term personality changes.[1] Moreover, this prevalent societal misconduct is mostly practiced by adolescents due to peer pressure and other societal factors.[1] Mounting evidences have shown that drug abuse generates neural problems as well as impaired reproductive functions.[2,3] Over the years, substance abuse disorders resulted for about 307,400 deaths which is fast becoming more alarming.

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A study carried out in an American high school showed that about 40 % of the seniors had used an illicit drug mostly marijuana in the last year followed by 33 % of the 10th graders and 15% of the 8th graders.[4] However, some of the drugs often abused are; amphetamines, cannabis, cocaine, methaqualone, tramadol and opioids which has led to numerous cases.[5] Furthermore, opioids are a widely prescribed class of medications for moderate to severe pain, but their use carries significant risks and side effects.[6] They achieve their analgesic effects by binding to opioid receptors in the central nervous system, blocking pain signals, and simultaneously triggering the release of large amounts of dopamine in the mesolimbic reward pathway.[7] This surge in dopamine activation in the pleasure centers reinforces the desired effects of the drug, leading to a strong potential for abuse and dependence.[8] Moreover, apart from the abuse of opioid drugs, psychoactive drugs are also misused commonly MDMA (3, 4-methylenedioxymethamphetamine) also known an ecstasy or molly because of its ability to produce experiences of emotional communion with a socializing effect. Which has proven to be the major cause of this abuse. It also leads to life threatening situations such as high blood pressure, seizures and panic attacks.[9] MDMA and other opioids have been documented to adversely affect the reproductive system.[2] MDMA-induced testicular toxicity is characterized by sperm DNA damage, tubular degeneration and interstitial edema in testes and also contribute significantly in affecting male reproductive hormonal levels.[2]Previous studies have consistently shown that MDMA and

Tramadol have a detrimental impact on spermatogenesis and Leydig cells. This leads to a decrease in spermatogenic cells, disruption of Sertoli cell tight junctions, DNA damage in sperm cells, tubular degeneration, and interstitial edoema in the testes.[2,10] MDMA and Tramadol have been found to have deleterious effects on the testes, causing changes in the DNA of spermatozoa and decreasing both the quality and quantity of semen.[2,3]FMRFamide is a neuropeptide with diverse regulatory roles in cellular functions. It possesses pharmacological actions, including anti-opiate effects.[11] Research demonstrates FMRFamide's ability to regulate cardiac activities through biochemical pathways by increasing cytoplasmic cAMP in the ventricular region. MDMA and Tramadol, despite their widespread use, are addictive substances with adverse effects on the central nervous system. They impair cardiac activity and present gonadal defects. Given the evident risks associated with MDMA and Tramadol abuse, it is crucial to explore the potential protective effects of FMRFamide (as an anti-opiate) against MDMA/tramadol-induced testicular toxicity in male Wistar rat models.

Materials and Methods

Animals

This research was conducted in the Animal House, Faculty of Basic Medical Science Babcock University, Ilishan-Remo, Ogun state, Nigeria. A total number of 30 male Wistar were used for this experiment (weighing from 160-170 g).

The experimental animals were housed and attended to in the Babcock University Animal House in accordance with the accepted guidelines for the care and use of animals in research and teaching, as established by the Institute of Laboratory Animal National Research. The experimental animals were given a week to acclimatise before the investigation began. The Babcock University Health Research Ethical Committee (BUHREC) granted ethical approval. The ethical clearance was granted and awarded the BUHREC NO: 768/19.

Materials

Plastic cages with an iron mesh to allow for cross ventilation, Pelletized food, Beddings (wood shavings), Feeding trough and water bottle, Marker for labeling, Oral cannula, Normal saline, Phosphate MDMA (Sigma-Aldrich (USA), Tramadol (Pfizer (USA), FMRFamide (Bachem (Switzerland), Heparinized bottles and Light microscope for semen analysis.

Preparation of Treatment Solution

Tramadol and MDMA were dissolved in normal saline and administered at 20mg/kg orally respectively to groups C and D while group E received co-administration of Tramadol and MDMA orally. The drugs were obtained from Sigma-Aldrich (USA).

Administration began immediately after one week of acclimatization. In the experimental study, the drugs were administered orally while FMRFamide was administered intraperitoneal (Table 1).

Measurement of Body Weight

The rat's body weight was monitored daily using a weighing balance for the whole period of treatment and stimulation. This was conducted to assess the changes in weight, whether it be an increase or decrease, within each group.

Mean Body Weight

The mean body weight was determined by subtracting the beginning body weight of the animals from their final body weight during the course of the experimental period, expressed as MBW = final body weight - initial body weight.

Animal Sacrifice

Following a 12-day therapy period, the sacrifice was performed by means of cervical dislocation. Subsequently, blood was extracted from the left ventricle using a needle and syringe, and then preserved in heparinized containers for hormonal analysis (namely, testosterone, follicle stimulating hormone, and luteinizing hormone). The obtained blood was then subjected to centrifugation at a speed of 3000 RPM for a duration of 5 minutes in order to separate the serum component. The rats were subsequently perfused with a solution of normal saline and 10% formaldehyde. The testes were surgically removed through a lower abdominal incision using a scalpel and forceps. They were then placed in sample bottles containing a 10% formaldehyde solution for histological investigation.

Semen analysis

The excised testes, which were not perfused, were placed in sample bottles filled with Phosphate-buffered saline (PBS). The epididymis was isolated by surgically removing it from the posterior region of the testes. Subsequently, the sperm cells were discharged onto a petri dish containing a solution of normal saline in order to undergo liquefaction. A little amount of this sperm solution was applied onto a glass slide, covered with a thin piece of glass, and seen using a light microscope for the purpose of analysing semen. This procedure was conducted following the method described by Taiwo et al. (2021).[12]

Hormonal Assay

The hormonal evaluation was conducted by obtaining a 2ml blood sample from the left ventricle of the heart and placing it in heparinized vials. The sample was then spun at a speed of 3000 revolutions per minute for a duration of 5 minutes using a Gulfex medical and scientific centrifuge from England. The plasma was isolated by the process of decantation and thereafter subjected to hormonal test for analysis.

Histopathological Examination

The testis was surgically removed, measured in weight, and preserved in a solution containing 10% formo-saline. Subsequently, the tissues were immersed in paraffin wax, cut into sections, and dyed with hematoxylin and eosin.

Statistical Analysis

The statistical analysis of the raw data was performed using the GraphPad Prism 5.0 programme. The data was transformed into grouped data and analysed using the one-way analysis of variance (ANOVA) method. Student Newman-Klaus was tasked with identifying the disparities between each mean, and a significance level of $p<0.05$ was used to determine if the discrepancies were statistically significant.

Results and Discussion

MDMA (3,4-methylenedioxymethamphetamine) and tramadol are widely misused central nervous system stimulants, commonly consumed for their psychoactive and analgesic properties. Recreational users often ingest doses of 50mg or more, which can lead to severe adverse effects such as seizures, faintness, and liver failure.[13,14] These substances are also known to induce significant reproductive toxicity, raising concerns about their impact on male fertility.

In the present study, adult male Wistar rats received oral administration of MDMA, tramadol, and a combination of MDMA/tramadol for 10 days to assess the toxic effects on male testicular reproductive function. Additionally, the rats received intraperitoneal administration of FMRFamide for two consecutive days to investigate its potential therapeutic effects on MDMA/tramadol-induced testicular toxicity.

FMRFamide is a neuropeptide with diverse physiological functions, including modulation of cardiovascular activities, pain responses, and reproductive processes. It has been found to exhibit anti-inflammatory properties, antioxidant activity, and the ability to modulate neurotransmitter systems involved in spermatogenesis. Given these attributes, FMRFamide presents a promising candidate for mitigating the reproductive toxicity induced by substances such as MDMA and tramadol.

This study aims to explore the protective effects of FMRFamide on testicular toxicity induced by MDMA and tramadol in a Wistar rat model, contributing to the understanding of its potential therapeutic

role in preserving male reproductive health amidst exposure to these harmful substances.

Table 1. Experimental Design

	No. of Rats	Treatment Schedule	Rationale
Group A	5	Normal Saline	Control
Group B	5	Intraperitoneal administration of FMRFamide at 2mg/kg for two consecutive days LD50:FMRfamide, 10mg/kg(Lingueglia *et al.*, 2006)	Positive control group for FMRFamide
Group C	5	Oral administration of Tramadol at 20mg/kg for 10 days LD50: Tramadol, 350mg/kg(Matthiesen *et al*., 1998	To induce effect of Tramadol
Group D	5	Oral administration of MDMA at 20mg/kg for 10 days. LD50: MDMA,350mg/kg(Goutarel, 1993)	To induce effect of MDMA
Group E	5	Oral administration of MDMA at 2omg/kg + Oral administration of Tramadol at 20mg/kg for 10 days	To induce damaging effect of MDMA and Tramadol
Group F	5	Co-administration of MDMA at 20mg/kg + Tramadol at 20mg/kg for 10 days with FMRFamide at 2mg/kg for two consecutive days	To mitigate effect of FMRFamide against MDMA and Tramadol

Body Weight and Testicular weight

The mean latency (g) for CON, FMRF, TRAM, MDMA, TRAM+MDMA and T+M+FMRF (202.40±3.93, 162.0±0.83, 159.40±1.47,136.40±1.749, 159.90±2.67, 163.60±1.83 respectively) initial body weight (g) showed a significant reduction when compared to their respective final body weight (g) (207.4±4.45,184.6±3.35, 160±4.83, 156.80±4.51, 182.20±2.76, 175.0±2.68) (Fig. 1). The mean latency for control (1.113±0.56) animal organ weight shows an obvious reduction which was not significant when compared to FMRF (1.30±0.80), TRAM (1.3 1±0.02), MDMA (1.06±0.06), MDMA+TRAM (1.30±0.02), T+M+FMRF (1.31±0.005) (Fig 2).

Importantly, initial and final body weight measurements of the adult male Wistar rats, as illustrated in Figure 1, exhibited a significant difference between the treated groups (FMRF, MDMA, TRAM+MDMA) and the control group (CON). This discrepancy in body weight could potentially be attributed to a loss of appetite induced by the daily dose of MDMA, as suggested by Lautieri (2019). MDMA, known for its appetite-suppressing effects, may have contributed to reduced food intake among the treated groups, consequently leading to a decrease in body weight over the course of the experimentation period. This indicates that while FMRFamide shows potential in mitigating MDMA-induced testicular toxicity, its effectiveness may be limited by the adverse effects on nutritional status caused by MDMA, highlighting the need for comprehensive therapeutic strategies that address both toxicity and associated side effects.

Semen Parameters

Sperm Morphology

The mean latency for control (56.67±6.36) animal sperm cell count (10^6cells/mls) shows an increase in sperm cells which was significant when compared to FMRF (26.00±10.58) and T+M+FMRF (19.00±2.64) and not significant when compared to MDMA (45.00±6.80), TRAM (39.67±4.09), TRAM+MDMA (34.33±2.02) (Fig 3)

The mean latency for control (44.67±7.42) animal sperm cell motility (%) shows an increase in sperm cell motility which was not significant when compared to MDMA (50.00±10.00) and a decrease when compared to FMRF (30.00±10.00), TRAM (33.33±8.81), TRAM+MDMA (40.00±11.55), T+M+FMRF (26.67±3.33) (Fig. 4).

Sperm Morphology

The mean latency for control (10.00±1.15) animal sperm cell morphology (%) shows increase in abnormal sperm cell morphology which was not significant when compared to FMRF (12.67±3.71), MDMA (16.67±4.41) and significant when compared to TRAM (31.67±4.41), TRAM+MDMA (37.33±1.45), T+M+FMRF (33.33±3.33) (Fig. 5).

The results from semen analysis, including sperm count (Figure 3), sperm motility (Figure 4), and sperm morphology (Figure 5), revealed noteworthy findings. Apparently, there was a significant difference in sperm count between the treated groups (FMRF, T+M+FMRF) and the control group (CON), with all treated groups exhibiting decreased sperm count compared to the control. This observation aligns with previous research by Marwa et al. (2014),[10] which reported impaired spermatogenesis function following tramadol administration. Notably, the decrease in sperm count across all groups further underscores the potential reproductive toxicity associated with the treatments administered in the study. In contrast, the analysis of sperm motility (Figure 4) indicated no significant difference between the groups, with all groups demonstrating a decrease in sperm motility. This finding implies that co-administration of MDMA and tramadol does indeed have an effect on sperm motility, consistent with previous studies,[15] which observed decreased sperm motility as a result of tramadol administration.

Meanwhile, analysis of sperm abnormal morphology, as depicted in Figure 5, revealed a significant difference between the treated groups and the control group (CON). Nevertheless, there was an observed increase in abnormal sperm morphology in the groups exposed to tramadol alone (TRAM), tramadol combined with MDMA (TRAM+MDMA), and tramadol combined with MDMA and

FMRFamide (T+M+FMRF), compared to the control group. Data obtained from this study aligns with previous research,[16] which demonstrated increased abnormal sperm morphology following tramadol administration at doses of 10mg and 20mg. Similarly, Sabour et al. (2017) reported increased abnormal sperm morphology following MDMA administration at a dose of 15mg.[17]

Figure 1: Effect of FMRFamide on adult male Wistar rat body initial and final weight changes of MDMA/Tramadol-induced testicular toxicity.
Values are represented as Mean±SEM. There was significant difference between CON and FMRF, CON and MDMA, CON and TRAM+MDMA

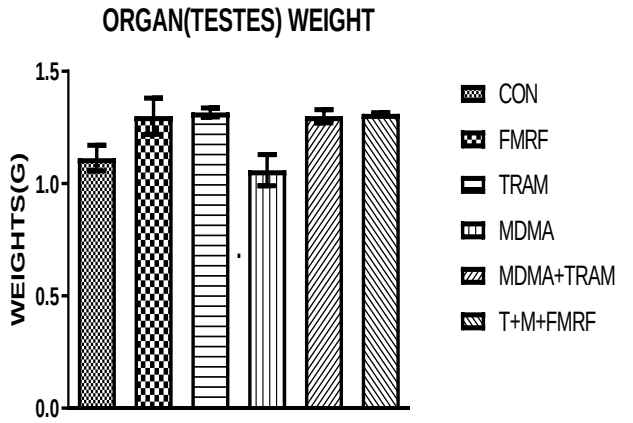

Figure 2: Effect of FMRFamide on adult male wistar rat testes weight in MDMA/Tramadol-induced testicular toxicity.
Values are represented as Mean±SEM. There was no significant difference between the groups.

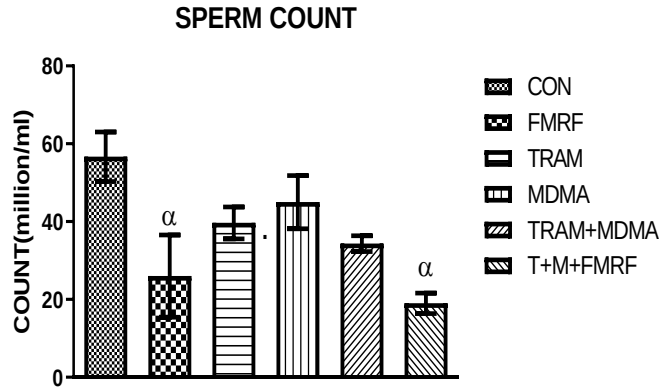

Figure 1: Effect of FMRFamide on sperm count of adult male wistar rat testes of MDMA/Tramadol-induced testicular toxicity.
Values are presented as Mean±SEM. There was significant difference between CON and FMRF, CON and T+M+FMRF

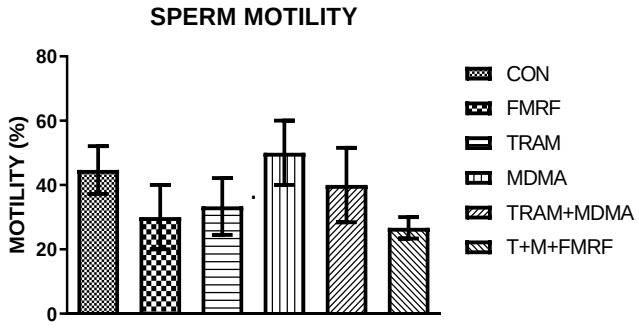

Figure 2: Effect of FMRFamide on sperm cell motility of adult male wistar rat testes in MDMA/Tramadol-induced testicular toxicity.
Values are presented as Mean±SEM. There was no significant difference between the groups

The increased incidence of abnormal sperm morphology in the tramadol-exposed groups suggests a potential detrimental effect of tramadol on spermatogenesis. Although, tramadol is known to act as a synthetic opioid analgesic, exerting its effects through the inhibition of serotonin and norepinephrine reuptake, as well as weak mu-opioid receptor agonism. Evidently, these mechanisms may disrupt the delicate balance of neurotransmitters and hormones involved in spermatogenesis, leading to abnormalities in sperm morphology. In addition, co-administration of MDMA and tramadol in the TRAM+MDMA group may likely exacerbate a derogatory toll on sperm morphology.
Importantly, MDMA is a psychoactive agent that can modulate serotonin release and inhibit its reuptake, leading to increased serotonin levels in the synaptic cleft. This alteration in serotonin signaling may further disrupt the hormonal milieu necessary for normal sperm development. Interestingly, the group receiving both tramadol, MDMA, and FMRFamide (T+M+FMRF) exhibited a higher incidence of abnormal sperm morphology compared to the control group but a lower incidence compared to the group exposed to MDMA and tramadol alone. This suggests that while FMRFamide was not entirely able to prevent the MDMA and tramadol-induced abnormalities in sperm morphology, it did exert a partial protective effect, reducing the severity of these effects. Notably, FMRFamide is a neuropeptide with diverse physiological functions, including modulation of reproductive processes. It is plausible that FMRFamide may exert protective effects against tramadol and MDMA-induced testicular toxicity through various mechanisms, such as its anti-inflammatory properties, antioxidant activity, and modulation of neurotransmitter systems involved in spermatogenesis.

Hormonal Assay
Luteinizing Hormone (LH)
The mean latency for control (14.53±2.26) animal luteinizing hormone (mIu/ml) level shows an increase in luteinizing hormone level which was not significant when compared to FMRF (9.70±1.90), TRAM (8.73±3.29), MDMA (4.93±0.13), TRAM+MDMA (13.73±2.57) and T+M+FMRF (13.83±2.45) (Fig 6)

Follicle Stimulating Hormone (FSH)
The mean latency for control (8.33±2.19) animal follicle stimulating hormone (mIu/ml) level shows an increase in Follicle stimulating hormone level which was not significant when compared to FMRF (19.76±5.02), TRAM (8.70±1.09), MDMA (7.0±0.58), TRAM±MDMA (11.47±4.64) and T+M+FMRF (16.20±4.64) (Fig. 7).

Testosterone
The mean latency for control (37.63±1.96) animal testosterone (ng/ml) level shows an obvious reduction in testosterone hormone level which was significant when compared to FMRF (13.63±3.68), and not significant when compared to TRAM (30.5±6.30), MDMA (32.37±0.40), TRAM+MDMA (24.90±5.56) and T+M+FMRF (27.3±6.90) (Fig 8)

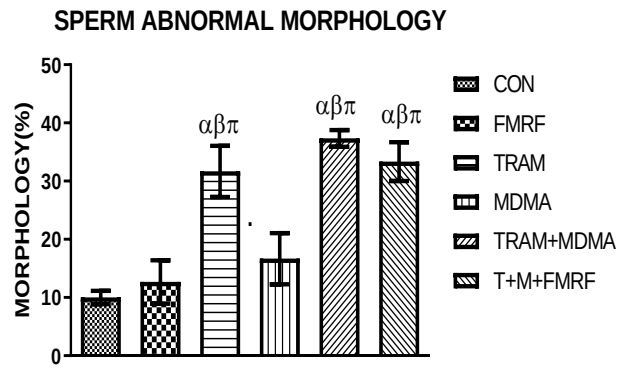

Figure 5: Effect of FMRFamide on sperm morphology of adult male testes in MDMA/Tramadol-induced testicular toxicity.
Values are presented as Mean±SEM. There was significant difference CON and TRAM, CON and TRAM+MDMA, CON and T+M+FMRF, FMRF and TRAM, FMRF and TRAM+MDMA, FMRF and T+M+FMRF, MDMA and TRAM, MDMA and TRAM+MDMA, MDMA and T+M+FMRF (P<0.05)

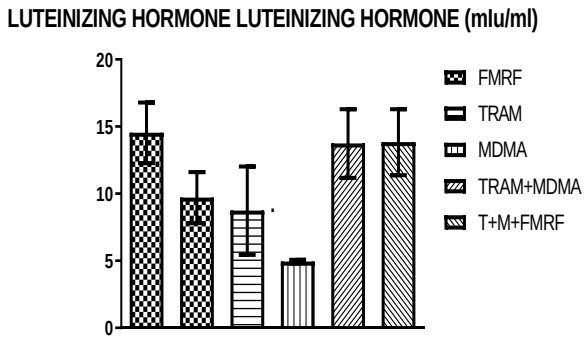

Figure 3: Effect of FMRFamide on Luteinizing Hormone (LH) levels in MDMA/Tramadol-induced testicular toxicity.
Values are presented as Mean±SEM. There was no significant difference between the groups (P<0.05)

The analysis of blood hormone levels, including luteinizing hormone (LH), follicle-stimulating hormone (FSH), and testosterone, provided valuable insights into the hormonal effects of the treatments administered in the study. Regarding LH and FSH levels, there were no significant differences observed between the treated groups and the control group. This suggests that the treatments may not have a pronounced effect on the secretion of these pituitary hormones responsible for regulating the function of the gonads.

However, the analysis of testosterone levels revealed a significant difference between the treated group receiving FMRFamide (FMRF) and the control group (CON). Remarkably, the treated groups exposed to tramadol alone (TRAM), MDMA alone, and the combination of tramadol and MDMA (TRAM+MDMA) showed a decrease in testosterone levels compared to the control group. This finding is consistent with previous research by Abdellatief et al. (2015) and Salah et al. (2020),[3,17] which demonstrated a decrease in testosterone levels following tramadol administration. Similarly, Dickerson et al. (2009) reported decreased testosterone levels following MDMA administration.

The observed decrease in testosterone levels in the treated groups suggests a potential disruption of the hypothalamic-pituitary-gonadal (HPG) axis, which regulates testosterone production. Testosterone plays a crucial role in maintaining male reproductive health, including the regulation of spermatogenesis, the development of secondary sexual characteristics, and the maintenance of libido. A decrease in testosterone levels can lead to impaired spermatogenesis, resulting in reduced sperm count, motility, and an increase in abnormal sperm morphology.

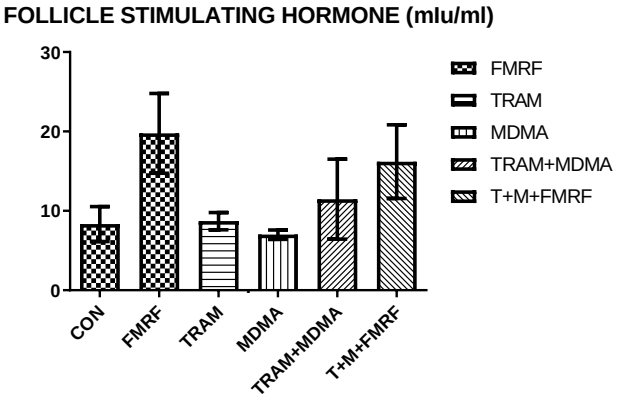

Figure 4: Effect of FMRFamide on Follicle Stimulating hormone levels in MDMA/Tramadol-induced testicular toxicity.
Values are presented as Mean±SEM. There was no significant difference between the groups (P<0.05).

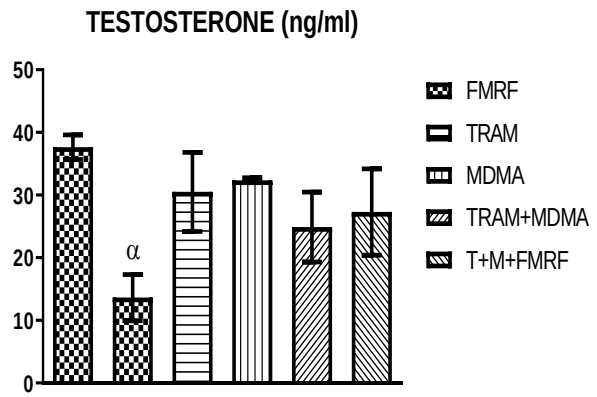

Figure 5: Effect of FMRFamide on Testosterone hormone levels in MDMA/Tramadol-induced testicular toxicity.
Values are presented as Mean±SEM. There was significant difference between CON and FMRF (p<0.05).

Tramadol and MDMA may interfere with the HPG axis through various mechanisms, such as inhibition of gonadotropin-releasing hormone (GnRH) release or direct suppression of Leydig cell function in the testes, leading to decreased testosterone production. The significance of the difference in testosterone levels between the FMRFamide (FMRF)-treated group and the control group suggests a potential protective effect of FMRFamide against the testosterone-suppressing effects of tramadol and MDMA. FMRFamide may exert its protective effects through the modulation of hormonal pathways or direct action on Leydig cells in the testes.

Histological Examination

In the control group interstitial cells (IC) are evenly distributed in the interstitial space (IS) and spermatogonium (SG) are well expressed in the seminiferous epithelium (SE). Spermatogonium (SG) is over expressed in mildly distorted seminiferous epithelium (SE) with uneven distribution of interstitial cells (IC) in interstitial space (IS) in FMRF, TRAM, MDMA, TRAM+MDMA, T+M+FMRF testicular sections (H and E X40) (Plate 1). Well demonstrated spermatogonium (SG) in seminiferous epithelium (SE) lined by evenly distributed interstitial cells (IC) in interstitial space (IS) in CON, FMRF. Seminiferous epithelium (SE) characterized by visible elongated spermatogonium (SG) lined by uneven distributed interstitial cells (IC) in interstitial space (IS) in TRAM, MDMA, TRAM+MDMA,

T+M+FMRF testicular sections (H and E X400) (Plate 2) Evenly distributed interstitial cells (IC) in interstitial space (IS) and spermatogonium (SG) are well expressed in the seminiferous epithelium (SE) in CON. Mild distortion of seminiferous epithelium with uneven demonstration of spermatogonium (SG) in FMRF, TRAM and T+M+FMRF lined by partial presence of interstitial cells (IC) in interstitial space (IS). Visible intense distorted seminiferous epithelium (SE) with uneven distribution of spermatogonium (SG) lined by partially visible interstitial cells (IC) in interstitial space (IS) in MDMA, TRAM+MDMA testicular sections (GORDON AND SWEET X100) (Plate 3).

Seminiferous epithelium (SE) containing well demonstrated spermatogonium (SG) lined by evenly distributed interstitial cells (IC) in interstitial space (IS) in CON, FMRF, TRAM+MDMA. Mildly distorted seminiferous epithelium (SE) with uneven distribution of spermatogonium (SG) lined by partial presence of interstitial cells (IC) in interstitial space (IS) in TRAM, MDMA, T+M+FMRF testicular sections (GORDON AND SWEET X400) (Plate 4). The histopathological assessment revealed several notable findings in the treated groups (TRAM, MDMA, TRAM+MDMA) compared to the control group. These included a decrease in sperm cell population, mild to severe distortion in seminiferous epithelium, decrease in interstitial cells in the interstitial space, and a decrease in sperm motility. These histological alterations are consistent with previous research documenting reductions in sperm cell population and motility due to the administration of MDMA and tramadol.[19,20,21]

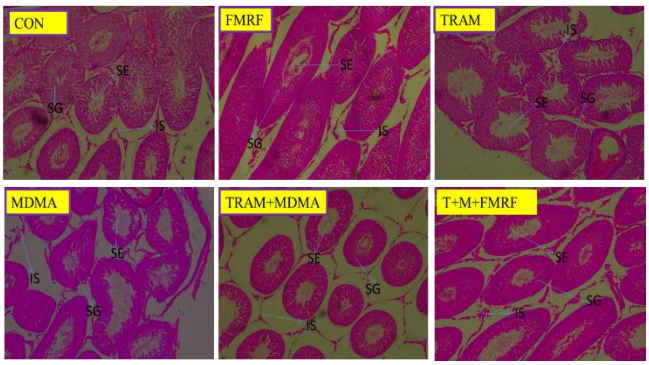

Plate 1: Photomicrographs of the testicular histomorphology of the adult male wistar rats across treated groups in low magnification.

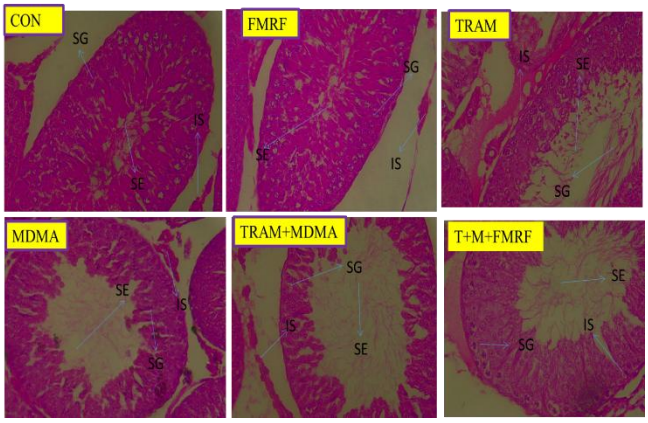

Plate 2: Photomicrographs of the testicular histomorphology of the adult male wistar rats across treated groups testicular sections.

Additionally, immunohistochemistry was employed to demonstrate the expression of Inducible Nitric Oxide Synthase (iNOS), a protein involved in the production of cellular nitric oxide. Previous studies have indicated that iNOS expression increases in cases of infections, chronic inflammations, and tissue damage, as it plays a role in the termination of microbes.[22,23] In this study, immunohistochemistry revealed expression of iNOS, appearing as a brown stain, in the control group (CON) which did not receive any treatment. However, the staining intensity of iNOS in spermatozoa and the interstitial space was higher in the treated groups FMRF, MDMA, and T+M+FMRF compared to the TRAM and MDMA groups. The increased staining intensity of iNOS observed in the treated groups indicates an increased expression or production of iNOS. Of particular note, the high staining intensity of iNOS in the treated group T+M+FMRF indicated immunopositivity, suggesting that FMRFamide may mitigate the deleterious effects of MDMA/tramadol-induced testicular toxicity[24, 25]. Apart from its role in infection, iNOS is also involved in nitric oxide production, which is a known vasodilator that can enhance sexual performance—a reason why tramadol is often abused. The authors have shown that FMRFamide would indirectly increase nitric oxide production. This increased nitric oxide production could have several implications. Firstly, increased nitric oxide production could improve blood flow to the testes, potentially enhancing nutrient delivery and waste removal. This improved blood flow might aid in mitigating the testicular toxicity induced by MDMA and tramadol. Secondly, while increased nitric oxide production could enhance sexual performance, it is essential to consider the balance required to avoid adverse effects associated with excessive nitric oxide, such as oxidative stress. Lastly, the increased nitric oxide production facilitated by FMRFamide may contribute to its gonadoprotective effects by reducing oxidative stress and inflammation, thus preserving testicular function and overall reproductive health.

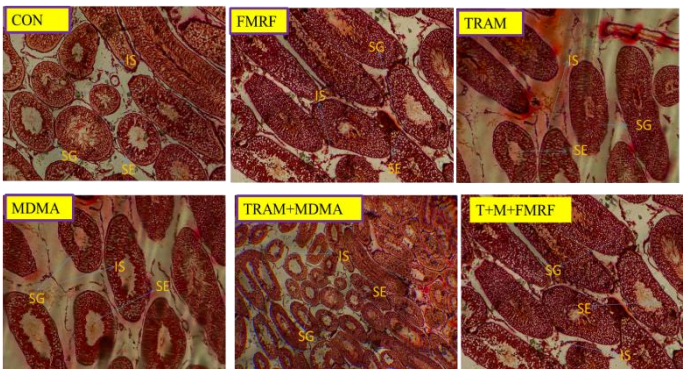

Plate 3: Photomicrographs of the testicular histomorphology of the adult male wistar rats across treated groups.

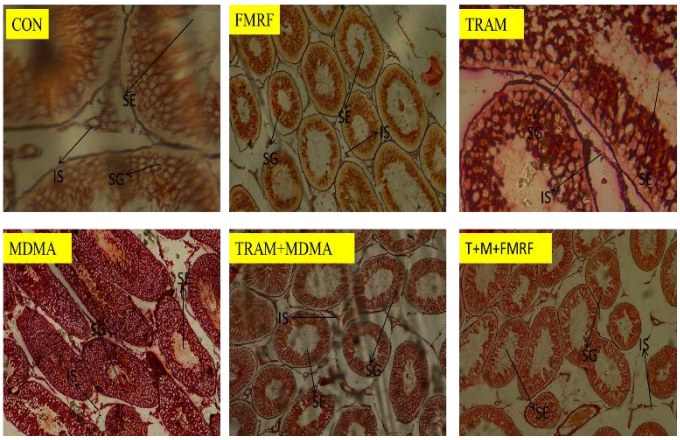

Plate 4: Photomicrographs of the testicular histomorphology of the adult male wistar rats across treated groups.

Conclusion

In conclusion, this study has demonstrated that the co-administration of MDMA and tramadol exerts detrimental effects on the testes, including reduced sperm count, motility, formation of abnormal sperm

cells, and hormonal dysfunction. These findings corroborate previous research indicating the reproductive toxicity of both MDMA and tramadol. Furthermore, the study highlights the potential therapeutic efficacy of FMRFamide in mitigating MDMA/tramadol-induced testicular toxicity. By curtailing histopathological alterations and modulating iNOS expression, FMRFamide shows potential as a viable therapy option for mitigating the negative impacts of MDMA and tramadol on reproductive health. However, it is crucial to note that further research is needed to fully understand the mechanisms underlying the protective effects of FMRFamide and to evaluate its safety and efficacy as a treatment for MDMA/tramadol-induced testicular toxicity. Continued investigation into FMRFamide and other potential interventions is essential for developing effective strategies to safeguard male reproductive health in the face of substance abuse.

Conflict of interest

The writers assert that they have no conflict of interest.

Authors' Declaration

The writers affirm that the work described in this article is original and that they will be responsible for any claims regarding the content of this article.

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