

## THE POSSIBLE AMELIORATIVE EFFECT OF PROPOLIS ON THE TESTES OF MALE ALBINO RATS WITH SUB-CHRONIC TRAMADOL ADMINISTRATION

BY

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### ABSTRACT

**Background:** Tramadol is an opioid analgesic which is utilized as a sexual stimulant and is consumed to manage severe pain. Tramadol usage is on the rise among Egyptian teenagers, it is one of the popular drugs of misuse. Abuse of tramadol can impact a number of bodily processes by causing oxidative stress. Propolis is a resinous compound that honey bees naturally create and has antioxidant benefits. **Aim of the work:** Studying the testicular effectiveness of sub-chronic tramadol use in male albino rats and the defensive function of Propolis. **Material and Methods:** Forty male albino rats were separated into four groups; GP I: negative control, GP II: Propolis treated, GP III: Tramadol treated, and GP VI received Tramadol plus Propolis for six weeks. All rats were examined for levels of Testosterone, Luteinizing hormone, Follicle stimulating hormone, and testicular markers for oxidative stress (Glutathione, Malondialdehyde & Superoxide dismutase). Histopathology, morphometry of testicular tissue, and sperm count were performed. **Results:** Tramadol treated group showed a considerable reduction in levels of gonadotrophic hormones, testicular Glutathione and Superoxide dismutase, while there was an increase in Malondialdehyde. Histopathology revealed normal histology of seminiferous tubules in GP I and II. At the same time, in GP III, the Vacuolation of Sertoli cells and atrophied seminiferous tubules. Administration of Propolis with Tramadol showed a worthy increase in levels of sexual hormones, Glutathione, Superoxide dismutase, and sperm count while a considerable lowering in Malondialdehyde with an improvement of the testicular histopathology. **Conclusion:** Propolis can be used as a protective agent against Tramadol-induced testicular toxicity.

**Keywords:** Propolis; Tramadol; Testis; Seminiferous.

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### INTRODUCTION

A synthesized 4-phenyl piperidine codeine analogue, tramadol is an eclectic agonist of  $\mu$  receptors with a lesser affinity for the  $\delta$ - and  $\kappa$ - opiate receptors. Additionally, it prevents the neuronal reuptake of serotonin and norepinephrine. Similar to other opioid-like medicines, it has the same side effects (dizziness, dry mouth, delayed ejaculation, hypotension, and itching). Tramadol, on the other hand, is less likely than morphine to cause respiratory depression, which is a deadly consequence of this class of medicines (Baghishani et al., 2018; Takzare et al., 2016).

It is advised to use tramadol in cases with drastic pain, especially after surgery, in a dose that should not exceed 400 mg. There is an increasing number of tramadol abusers globally, and the health risks of tramadol typically come with its misuse for a prolonged length of time (Nazarzadeh et al., 2014). Tramadol misuse was frequent

amongst Egyptian teenagers, with more than one-third having drug-related difficulties (Abdel Wahab et al., 2018).

The consumption of sexual enhancers is common among young men who do not have erectile dysfunction. Opioids has become very popular for a variety of reasons, including the need to increase sexual drive, the inclination to obtain a more rigid and enduring erection, need for higher coital frequency, and preparedness to postpone emission of semen (Nna et al., 2016).

Endogenous opioids are essential for physiological sexual function. They bind to certain receptors to aid in regulating the production of gonadotropin-releasing hormone and, consequently, follicle-stimulating hormone (FSH) and luteinizing hormone (LH), which in turn activate the somatic cells that assist in spermatogenesis straightforwardly in the testes. Morphine decreases LH release and lowers testosterone levels, which impairs testis responsibility.

Opioid usage has been associated to hypogonadism, low libido, and infertility (Vuong *et al.*, 2010).

Tramadol has been investigated as a possible treatment for premature ejaculation. Multiple clinical trials indicate better intravaginal ejaculatory latency time with various tramadol dosages used on a regular or on-demand basis. Its effectiveness might arise from anti-nociceptive and anesthetic actions, modulating the central system by blocking serotonin and norepinephrine reuptake. However, before prescribing this prescription, the likelihood of addiction and other consequences on sexual performance ought to be adequately evaluated (Hisasue, 2016).

Tramadol abuse can harm the male reproductive system by lowering sex hormones, altering the shape of sperm, and diminishing healthy sperm levels. The establishment of oxidative stress and apoptosis within spermatocytes is one of the mechanisms of tramadol-persuaded testicular injury (Ibrahim and Salah-Eldin, 2019).

The disproportion in the formation of free radicals and the antioxidative shielding system is mentioned as oxidative stress. This means that the body's defense mechanisms are unable to neutralize the free radicals created; this can result in mitochondrial malfunction and apoptosis, which harms tissue cells (Schulz *et al.*, 2014).

Propolis is a naturally existing resinous compound created by honey bees and obtained from buds, leaves, and other vegetal elements. It contains numerous biochemical components with a variety of biological and pharmacological effects, including polyphenols, flavonoids, aglycones, phenolics, and ketones. Greek practitioners of folk medicine used raw propolis to improve gastrointestinal functions, anti-ageing, anti-fatigue, and other benefits (Mahmoud and Elsoadaa, 2013; Simone-Finstrom and Spivak, 2010).

Because of its anti-oxidative and anti-inflammatory qualities, propolis possesses gastro-protective, immune - modulatory, tissue regeneration, and antineoplastic activities (Kurek-Gorecka *et al.*, 2013). Propolis' shielding aptitude results from its adjusting effect on the antioxidative enzymes,

which prevents the formation of free radicals and minimizes subsequent damage (Barlak *et al.*, 2015).

### AIM OF THE WORK

The aim of the current study was to detect the possible ameliorative efficiency of propolis on the sub-chronic consequences of tramadol administration on testes of male albino rats.

### MATERIAL AND METHODS

#### • Chemicals:

- Tamol (Tramadol HCl), 200 mg tablets, were gained from Hikma pharma. Co., Egypt. According to a wide range of literature search and different preliminary studies there were wide range of tramadol dosing can cause different manifestations. An overdose of tramadol cannot be precisely measured in milligrams. Individual tolerance and genetics are linked to the amount of tramadol needed to overdose (Nakhaee *et al.*, 2021). So the used dose of tramadol was chosen based on Matthiesen *et al.* (1998) who concluded that LD50 of Tramadol in rats is 300 mg/kg. As a result, the 150 mg/kg (half dose of LD50) tramadol dose used in this study was intended to mimic sub-chronic toxicities.

Tramadol tablets were dissolved in sterile distilled water and filtered through gauze to get a clear solution of the desired concentration.

**Propolis;** after being frozen, propolis was taken from honeybee hives on an Egyptian farm. Ten grams were powdered and added to 100 milliliters of sterile distilled water. To obtain the clear working solution, the mixture was heated for one hour while frequently shaken, allowed to cool, and filtered through gauze (Sforcin and Bankova, 2011).

- The dose of Propolis (200 mg/kg/day) was selected according to Bhadauria (2012) and many other studies.

#### **Experimental animals and Study design:**

At the start of the experiment, forty fully mature male albino rats weighing 180-200 grammes were bought from the National Research Centre breeding unit (Giza, Egypt). All rats in metal frame cages were kept at a constant temperature (21-24°C), 50-60% relative humidity, and a 12-hour light-dark cycle. They were provided with a typical balanced meal and had unrestricted access to running tap water. The Ethical Committee of

Fayoum University's College of Medicine authorized the full experimental procedure, including animal usage. It was governed by institutional and national rules for animal care and utilization (Aldemir et al., 2014). The rats were split into four groups of ten each:

- **G I:** Considered as negative control group.
- **G II:** Rats received propolis (200 mg/kg/day).
- **G III:** Rats given tramadol (150 mg/kg/day).
- **G IV:** Propolis (200 mg/kg/day) and tramadol (150 mg/kg/day) were given to rats.

All drugs have been administered by orogastric gavage once a day for six weeks to detect the sub-chronic effect of tramadol on testis.

#### **Blood sampling and measurement of testosterone, FSH, and LH:**

After anesthesia with sodium thiopental, the rats were sacrificed, and blood was obtained from the ocular sinus and instantly placed in heparinized tubes, then centrifuged and retained at  $-20^{\circ}\text{C}$ . The levels of testosterone, luteinizing hormone (LH), and Follicular stimulating hormone (FSH) in serum were quantified via ELISA kits from (LSBio Company) and the manufacturer's instructions.

#### **Tissue preparation for histopathological and morphological examination of the testis:**

After anesthetic, the animals were slaughtered via cervical decapitation. The testis was subsequently removed through laparotomy, cleaned with ice-cold saline buffer, wiped in filter sheets, and divided into two portions. The first section was submerged in 10% formalin for histological studies before being dyed with hematoxylin and eosin (H&E).

The epithelium height and mean diameter of the seminiferous tubules were measured using image-analysis software (Leica Q 500 MCO, Germany). H&E-stained slices were utilized to measure the above parameters at a magnification  $\times 200$ .

#### **Analyses of oxidative stress markers and antioxidants:**

Each rat had one gram of testicular tissue removed and tested for Malondialdehyde

(MDA), Glutathione Reduced (GSH), and Superoxide Dismutase (SOD), by colorimetric method utilizing commercial kits from (BIODIAGNOSTIC Company) and following the industrialist instruction.

#### **Sperm collection and examination**

The caudal epididymis was slit with the point of surgical scissors for sperm inspection, and a tiny quantity of sperm fluid pouring out was gathered. Using one ml chambers, sperm heads were counted.

#### **Statistical analysis:**

SPSS version 28 in Windows 10 was used to collect data for statistical analysis. Mean and standard deviation are used to describe quantitative data. To compare the groups, ANOVA statistical analysis was utilized.  $p < 0.05$  values were deemed significant statistically.

## **RESULTS**

**Table (1)** Demonstrated considerably lower levels of Testosterone, FSH, and LH in Tramadol-treated group (group III) than in the control group. Concomitant use of Propolis with Tramadol in group IV revealed a worthy increase in serum levels of these hormones compared to group III. Concerning oxidative stress-related tissue markers, the Tramadol-treated (group III) recorded a worthy elevation in testicular MDA, along with low testicular GSH and SOD than in the control group. The combination of propolis and tramadol (group IV) resulted in a considerable diminution in testicular MDA and a rise in GSH and SOD compared to group III, as can be seen in **Table (2)**. **Table (3)** demonstrated a considerable lowering in sperm number in group III compared to group I. In contrast, with the addition of propolis (group IV), a worthy increase in sperm count was noted compared to group III.

#### **Histopathology:**

Microscopic examination of testis from the group I (**Fig. 1-2**) revealed normal histology of seminiferous tubules, which were consistent in size and shape. Germinal cells and pillared Sertoli cells lined the tubules. The germinal cells were set in layers from the basement membrane to the tubule lumen.

Regarding group II (**Fig. 3-4**), the seminiferous tubules showed the same normal appearance like GI with presence of numerous spermatids.

Regarding group III (**Fig. 5-8**), the testicular tissue showed distinct histopathological alterations. Vacuolation of Sertoli cells was

frequently detected within the affected seminiferous tubules. Atrophied seminiferous tubules were characterized by contraction or disconnection of basement membrane, and disrupted spermatogenic layers.

Exfoliation of germ cells was frequently detected, illustrated by sloughing of germ cells into the lumen of seminiferous tubules. Vascular congestion with significant edema was evident in the interstitial tissue.

Histopathology of sections from group IV (Fig.

9- 11) showed an improvement of the testicular tissue, which appeared as mild exfoliated germ cells into the somniferous tubular lumen. A sporadic case showed limited interstitial edema. Several examined sections exhibited apparently normal seminiferous tubules.

These alterations between groups were validated by morphometric analysis (Fig. 12-13), which revealed that the mean of tubular diameter and epithelial height in group III had significantly lower values in comparison to the other groups.

**Table (1): Statistical analysis of the effects of Tramadol, Propolis and their combination on testosterone,FSH & LH using one way ANOVA: (N=40)**

Parameter	Group	Mean	± SD	F	P- value
Testosterone (ng/ml)	Group I	6.92	0.05888	1926.023	<.001*
	Group II	7.669	0.13876		
	Group III	2.557	0.25945		
	Group IV	5.123	0.13158		
FSH (ng/ml)	Group I	4.441	0.15014	687.08	<.001*
	Group II	5.002	0.11419		
	Group III	1.973	0.09044		
	Group IV	3.215	0.24919		
LH (ng/ml)	Group I	4.971	0.33235	633.279	<.001*
	Group II	5.054	0.18798		
	Group III	1.262	0.17706		
	Group IV	3.074	0.16688		

Group I: Negative control  
SD: standard deviation

Group II: Propolis

Group III: Tramadol

Group IV: Propolis and Tramadol

\* P- value <.001= high significant

**Table (2): Statistical analysis of the effects of Tramadol, Propolis and their combination on testes oxidativemarkers of rats using one way ANOVA:**

		Mean	± SD	F	P- value
MDA (mmol/g tissue)	Group I	0.53	0.04216	773.068	<.001*
	Group II	0.384	0.02319		
	Group III	3.619	0.28203		
	Group IV	1.162	0.18671		
GSH (mmol/g tissue)	Group I	3.823	0.12499	1592.159	<.001*
	Group II	3.275	0.09455		
	Group III	0.989	0.08089		
	Group IV	2.015	0.09823		
SOD (U/g tissue)	Group I	2.739	0.0962	654.277	<.001*
	Group II	3.052	0.15483		
	Group III	0.819	0.03872		
	Group IV	1.614	0.17367		

Group I: Negative control

Group II: Propolis

Group III: Tramadol

Group IV: Propolis and Tramadol

MDA: Malondialdehyde

SD: standard deviation

\* P- value < 001= high significant

**Table 3: Statistical analysis of the effects of Tramadol, Propolis and their combination on sperm count of male albino rats using one way ANOVA (N=40)**

		Mean	± SD	F	P- value
Sperm count (10 <sup>6</sup> /mL)	Group I	46.13	1.19722	1296.141	<.001*
	Group II	52.32	1.88562		
	Group III	28.64	1.7127		
	Group IV	39.12	1.49071		

Group I: Negative control

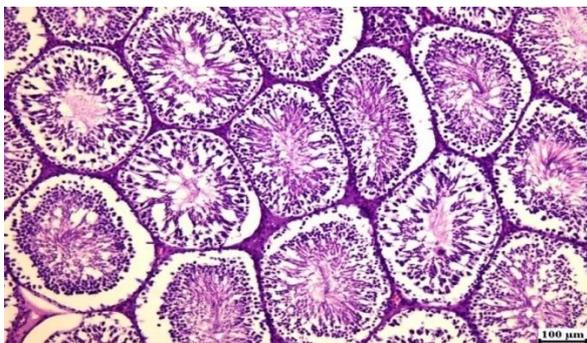
Group II: Propolis

Group III: Tramadol

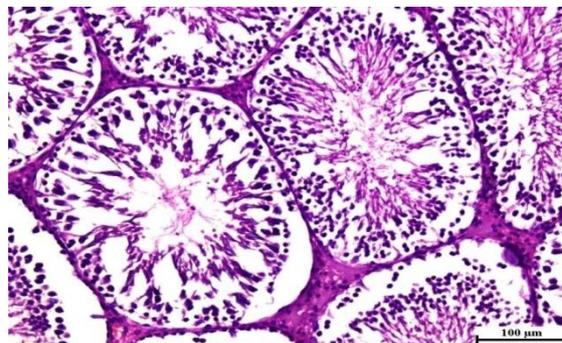
Group IV: Propolis and

Tramadol SD: standard deviation

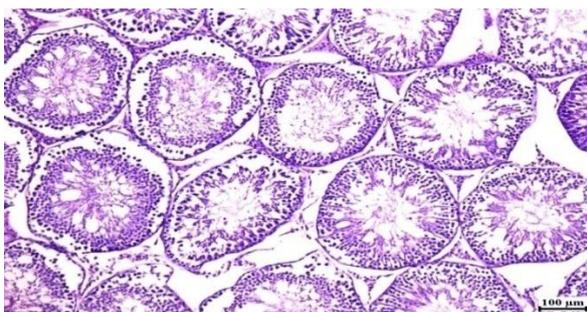
\* P- value <.001= high significant



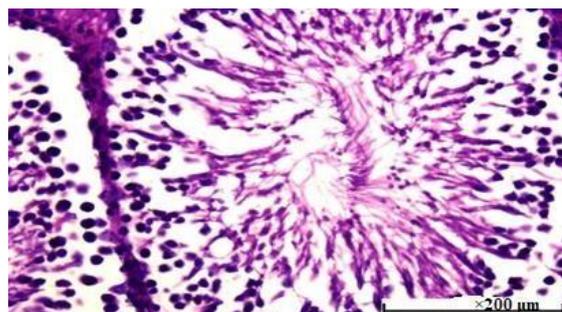
**Fig. (1)** Photomicrograph of testes, G I group higher magnification showing normal seminiferous tubules (H&E).



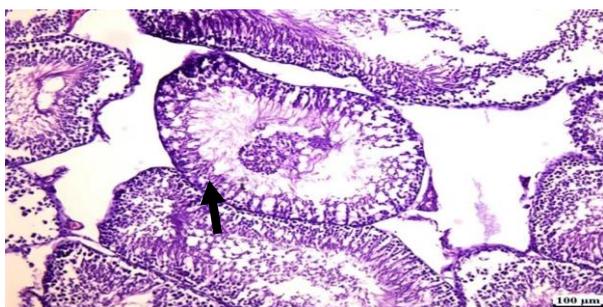
**Fig. (2)** Photomicrograph of testes, G II group higher magnification showing normal seminiferous tubules (H&E).



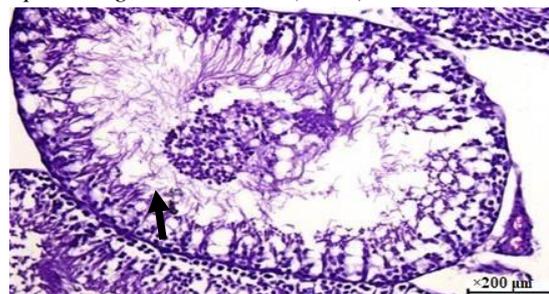
**Fig. (3)** Photomicrograph of testes, G II group showing normal seminiferous tubules (H&E).



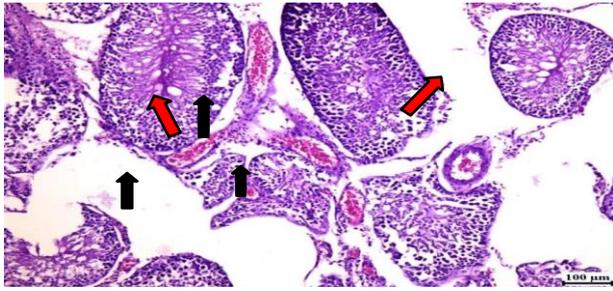
**Fig. (4)** Photomicrograph of testes, G II group higher magnification showing normal spermatogonial cells (H&E).



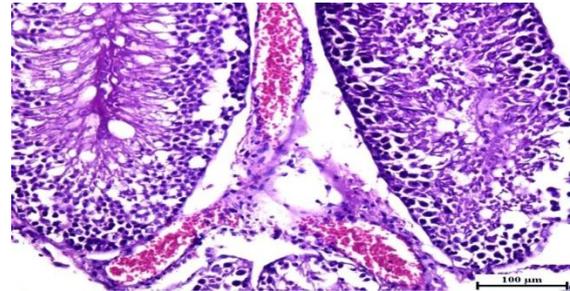
**Fig. (5)** Photomicrograph of testes, G III group showing exfoliated germ cells into the lumen of seminiferous tubule (arrow) (H&E).



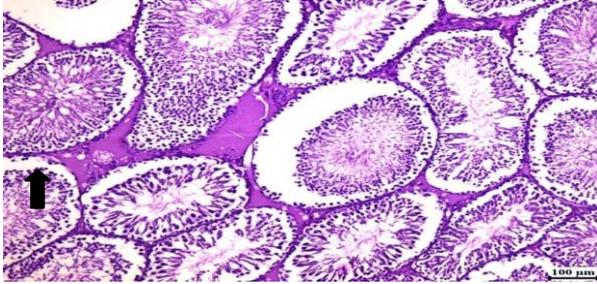
**Fig. (6)** Photomicrograph of testes, G III group showing exfoliated germ cells into the lumen of seminiferous tubule (arrow) (H&E).



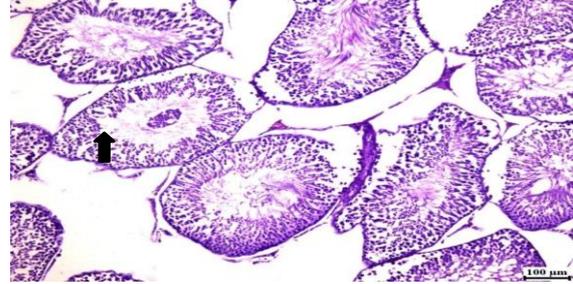
**Fig. (7)** Photomicrograph of testes, G III group showing marked congestion of the interstitial blood vessels (black arrows). Note vacuolation of Sertoli cells (red arrows) (H&E).



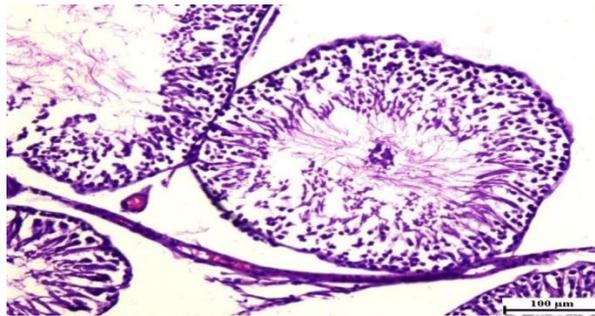
**Fig. (8)** Photomicrograph of testes, G III group higher power showing marked congestion of the interstitial blood vessels (H&E).



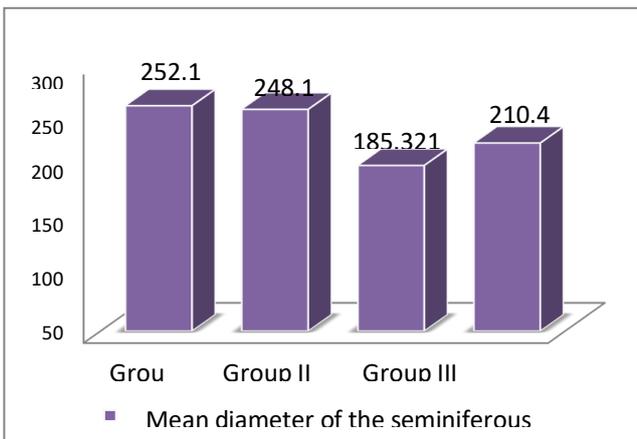
**Fig. (9)** Photomicrograph of testes, G IV group showing mild interstitial edema (arrow) (H&E).



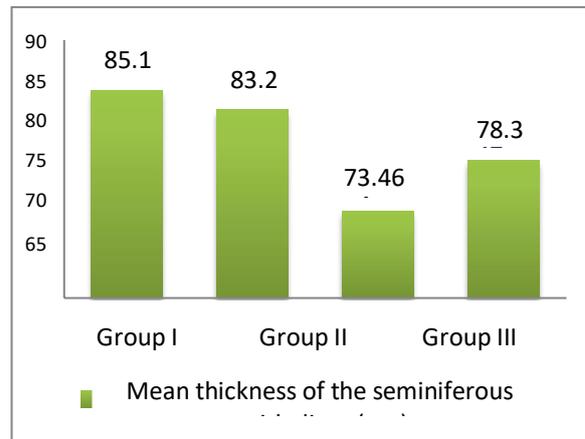
**Fig. (10)** Photomicrograph of testes, G IV group showing mild exfoliated germ cells (arrow) (H&E).



**Fig. (11)** Photomicrograph of testes, G IV group higher power showing mild exfoliated germ cells (H&E).



**Fig. (12):** Mean diameter (mean  $\pm$  SD) of the seminiferous tubules ( $\mu\text{m}$ ) in the studied groups. \*: significant difference compared to group I.



**Fig. (13):** Mean thickness (mean  $\pm$  SD) of the seminiferous epithelium ( $\mu\text{m}$ ) in the studied groups. \*: significant difference compared to group I.

## DISCUSSION

In recent years, sex-enhancing drugs have risen to the top of the list of medications that are both prescribed and *abused* (Nna *et al.*, 2016).

The current study revealed that rats received tramadol for six weeks showed a considerable diminution of Testosterone, LH, and FSH levels, which agrees the results observed by Babaei *et al.*, (2012) and Salah *et al.*, (2020). The reduction of LH and FSH was cleared up by Bliener *et al.*, (2005) and Pimpinelli *et al.*, (2006) who demonstrated that greater prolactin levels achieve the opioid influence on GnRH; nonetheless, increasing prolactin production may directly inhibit testosterone synthesis.

Opioids are widely recognized for adjusting the cell function of the hypothalamus, pituitary, and testis. Gonadal hormones influence endorphinergic neurons in the hypothalamus, and opioid receptors were identified in refined gonadotrophs. Opioid involvement in the natural pulsatility of GnRH flow at the hypothalamic level has been demonstrated, resulting in a reduction in FSH and LH output from the pituitary and testicular testosterone production (Aloisi *et al.*, 2009).

The current results revealed that tramadol supplementation in adult male rats for six weeks produced oxidative damage in the testes, mainly by lipid and protein oxidation, with lower GSH, superoxide dismutase levels, and sperm count while increased Malondialdehyde in testicular tissue versus the control group. These findings corresponded with those of Koohsari *et al.*, (2020) who found that tramadol-treated groups had lower GSH levels (Safarinejad *et al.*, 2013) discovered a substantial drop in SOD activity and increased spermatozoa DNA fragmentation in the sperm of opioid users. Similarly, El-Gaafarawi (2006) concluded a worthy rise of MDA levels in tramadol-treated rats.

The major antioxidant system in sperm is composed of superoxide dismutase, catalase, and glutathione (GSH). These metalloenzymes can be found both within and outside the cell. Seminal SOD activity has been highly associated with sperm

concentration. GSH peroxidase is concerned in catalysis the diminution of hydrogen peroxide and organic peroxides, lowering lipid peroxidation and improving sperm membrane properties (Yan *et al.*, 2014).

Tramadol considerably increases oxidative stress markers. Tramadol also provoked testicular mitochondrial impairment by causing the mitochondrial membrane potential to collapse and swell (Koohsari *et al.*, 2020).

Tramadol combines with molecular oxygen, triggering a chain reaction that creates free radicals such as hydrogen peroxide and superoxide. It can disrupt antioxidant enzymes in several organs, including the testis, culminating in spermatogenic cell death (Ghoneim *et al.*, 2014). The histopathology and the morphometry of the testicular tissue revealed that the tramadol-treated group had an increased number of atrophied seminiferous tubules with a marked reduction in spermatogenesis and frequent vacuolation of Sertoli cells. Sloughing germ cells inside the lumen of seminiferous tubules was found; also, blood vessels within the testicular tissue were markedly congested. Similarly, Abdellatif *et al.*, (2015) and (Salah *et al.*, 2020) had identified the same results. Increased ROS

combined with diminished antioxidant defense, causes redox imbalances and DNA damage in the sperm. Spermatozoa are particularly vulnerable to the devastating impacts of ROS because of the large concentrations of unsaturated fatty acids in cell membranes. ROS accelerate lipid peroxidation, contributing to intracellular oxidative stress. The process involves lipid peroxidation, membrane integrity loss with increased permeability, structured DNA damage, and apoptosis (Schuppe *et al.*, 2008).

Spermatozoa are permanently exposed to the "oxygen paradox"; a disequilibrium between the reactive oxygen species and antioxidative ending with much ROS formation, which is implicated in male infertility (Maneesh and Jayalekshmi 2006).

Because spermatozoa have small antioxidant content, they are especially vulnerable to the negative consequences of ROS.

Furthermore, oxidative stress is induced by excessive amounts of ROS, most prevalent in organs with limited antioxidant activity, like testicular tissues (*Sheweita et al., 2018*).

Antioxidants can potentially mitigate or prevent cellular injury caused by reactive oxygen species (ROS). Propolis contains extensive pharmacological activities, including anti-inflammatory, anti-cancer, and antioxidant; propolis was subsequently identified as an efficient ROS scavenger (*Ozguner et al., 2005*).

In their studies, *Abu-Almaaty et al., (2019)* and *El-Amawy et al., (2021)* proved that Propolis has an antioxidant function versus oxidative stress and organ damages incurred by Aluminum Silicate and heavy metals, respectively, and demonstrated that Propolis has a protective impact on various organs of albino rats.

The current findings revealed that Propolis co-administration minimizes tramadol-induced changes in testicular oxidant balances and induces a substantial reduction in MDA while increasing SOD and GSH levels. When compared to the tramadol-treated group, it also improved gonadotropin levels and sperm count. Similarly, *Abdelrazek et al., (2020)* concluded the same results.

The benefits of propolis as an antioxidant were evident, where there were improved seminiferous tubules histopathological and morphometric findings in group VI, as previously mentioned.

*Hashem, (2021)* investigated the shielding role of propolis against testicular injury triggered by carbon tetrachloride (CCl<sub>4</sub>) and discovered that propolis boosts testosterone levels while diminishing oxidant levels. In a group that consumed propolis together with CCl<sub>4</sub>, histopathology revealed a standard structure of seminiferous tubules, minor congested testicular blood vessels, and substantial dynamic spermatogenesis.

*Yousef et al., (2010)* discovered the antioxidant benefits of propolis in rabbits treated with triphenyltin, which coincided with histological data that demonstrated the conventional architecture of testes in the propolis-treated group.

The antioxidant effectiveness of propolis is

connected to its propensity to scavenge free radicals, which shields spermatozoa from the negative consequences of oxidative stress and diminishes lipid peroxidation (*Moustafa et al., 2014*).

## CONCLUSION

Tramadol is widely used among addicts, and it can produce a depressant effect on testicular function as lowering serum Testosterone level, FSH, LH, and sperm count. Also, it can alter the normal pattern of seminiferous tubules. Propolis, as an antioxidant, can counter-act the harmful consequences of tramadol on testes.

## RECOMMENDATIONS

Oral Propolis can be used to recover the Tramadol-induced damage in testicular tissue.

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## التأثير التحسيني المحتمل للبروبوليس على خصية ذكور الفئران البيضاء مع جرعات الترامادول شبه المزمنة

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## المخلص العربي

الترامادول هو مسكن أفيوني مركزي المفعول يستخدم عادة كمنشط جنسي ويتم استهلاكه لإدارة الألم المتواضع إلى الشديد. استخدام الترامادول أخذ في الارتفاع على مستوى العالم، وبين المراهقين المصريين، يعد أحد أشهر عقاقير إساءة الاستخدام. يمكن أن يؤثر تعاطي الترامادول على عدد من العمليات الجسدية من خلال التسبب في الإجهاد التأكسدي والاستماتة البروبوليس هو مركب راتنجي يخلقه نحل العسل بشكل طبيعي وله فوائد مضادة للأكسدة.

**الهدف:** الهدف هو دراسة فعالية الخصية لاستخدام الترامادول شبه المزمن في ذكور الفئران البيضاء والوظيفة الدفاعية المحتملة لبروبوليس.

**الطريقة:** تم فصل أربعين من ذكور الجرذان البيضاء الناضجة إلى أربع مجموعات ؛ المجموعة الأولى :مجموعة التحكم السلبية، المجموعة الثانية :تم اعطائها البروبوليس ، المجموعة الثالثة :تم اعطائها الترامادول ، المجموعة الرابعة :تم اعطائها الترامادول مع البروبوليس لمدة ستة أسابيع .تم فحص جميع الفئران بحثاً عن مستويات هرمون التستوستيرون، وهرمون تحفيز التحوصل ، الهرمون الملوتن ، وعلامات الخصية للإجهاد التأكسدي ( الجلوتاثيون ، مالونديالدهيد وسوبروكسيد ديسموتاز) وتم إجراء التشريح المرضي وتشكيل أنسجة الخصية.

**النتائج:** أظهرت مجموعة الترامادول المعالجة انخفاضاً كبيراً في مستويات هرمونات التغذية الغازية والجلوتاثيون الخصي وديسموتاز سوبرأكسيد، بينما كانت هناك زيادة في مالونديالدهيد .كشفت الفحص المرضي النسيجي عن الأنسجة الطبيعية للأنايبب الحلقية في المجموعة الأولى و الثانية .وفي الوقت نفسه، في المجموعة الثالثة، تميزت بفرغ خلايا سيرتولي والأنايبب الحلقية الضامرة بانقباض أو فصل الغشاء القاعدي الأنبوبي .أظهر إعطاء البروبوليس مع الترامادول زيادة جديرة بالاهتمام في مستويات الهرمونات الجنسية، والجلوتاثيون وفوق الأكسيد، وانخفاضاً كبيراً في مالونديالدهيد مع تحسين أمراض أنسجة الخصية.

**الاستنتاج:** يمكن استخدام البروبوليس، كمضاد للأكسدة، كعامل وقائي ضد سمية الخصية التي يسببها الترامادول.

**الكلمات الرئيسية:** البروبوليس ؛ ترامادول ؛ الخصية ؛ مثير للاهتمام.