THE ROLE OF PROPOLIS ON REPRODUCTIVE FUNCTIONS OF
SELECTIVE SEROTONINE REUPTAKE INHIBITORS (SSRIs)-INDUCED SEXUAL
DYSFUNCTION IN MALE WISTAR RATS

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Dedication

This research work is dedicated to the **ALMIGHTY ALLAH, THE BENEFICENT, THE MERCIFUL, THE IMMORTAL, THE INVISIBLE, THE GIVER AND TAKER OF LIFE** who spared my life to see the completion of this programme amidst several hurdles and uncertainties. “**Alhamdulillah Rabil haalamin**”
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<td>Nitric oxide</td>
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Abstract

**Background:** Several studies have found a link between medicinal plants to produce definite physiological actions in the body systems. Many products are being exhibited as "natural" sexual enhancer, but no clinical trials or scientific studies have been documented to support their effectiveness for the treatment of erectile dysfunction (ED). Out of curiosity, propolis (PL) which is a mixture collected by honey bees from parts of plants, buds, exudates, tree buds, sap flows is indeed attracting much research interests. Especially, now being locally consumed for sexual enhancement. It is estimated that up to 50-80% of patients that take selective serotonine reuptake inhibitors (SSRIs) suffer from post-SSRIs sexual dysfunction (PSSD) of one degree or another PSSD is a frustrating situation that affects the self esteem of victims. The objective of the present study was aimed at exploring and evaluating the medicinal activities of propolis using animal model with the view to justify the ethnomedicinal claim of its indigenous usage to be beneficial in improving or treating various sexual dysfunctions.

**Methods:** Sexually potent male Wistar rats weighing between 140g–190g, and equal number of sexually unexposed female (120g-130g) were used for this study. Sexual dysfunction was induced in the male rats by administration of paroxetine for two weeks, and confirmed by sexually exposing them to female rats. They were randomly divided into eight groups of six rats each (n=6), and were either left with the vehicle alone (0.9% NaCl), or administered sildenafil, or administered graded concentrations of PL, or co-administered sildenafil and propolis orally over two different durations of 7 days and 60 days. At the end of these days, copulatory behaviour test was done overnight to visually assess sexual indices (MF, IF, EF, ML, IL, EL and PEI) following which the animals were sacrificed for semen collection for seminal fluid analysis, and testes isolated for histological assessment. In addition, blood samples were collected from
animals administered for 60 days for biochemical analysis of LH, FSH, Testosterone and Nitric oxide.

**Results:** Copulatory activity on day 7 revealed that high dose propolis and sildenafil sustained all copulatory frequencies generally, except EF that was significantly (p<0.05) reduced by sildenafil citrate, while all these were decreased significantly (p<0.05) in paroxetine untreated rats. In the same vein, high dose propolis and sildenafil sustained all copulatory latencies except IL that was significantly increased, while all were significantly (p<0.05) increased in paroxetine untreated rats. The PEI was also sustained by high dose propolis and sildenafil.

Copulatory activity on day 60 showed that propolis sustained, and increased significantly (p<0.05) all copulatory frequencies. Sildenafil also sustained all the copulatory frequencies except IF which was significantly (p<0.05) decreased, while all the latencies were sustained, and significantly (p<0.05) reduced. Sildenafil however, globally significantly increase all the copulatory latencies. The PEI was also sustained by high dose propolis and sildenafil citrate.

Semen analysis on day 7 revealed a significant (p<0.05) increase in sperm count in high dose propolis administration, while there was significant (p<0.05) decrease in sildenafil and paroxetine untreated rats. The sperm motility was also sustained by high dose propolis while it was significantly (p<0.05) reduced in sildenafil and paroxetine untreated rats. The sperm total abnormality was also reduced by high dose of propolis. While sildenafil sustained the total abnormality, paroxetine untreated animals caused significant (p<0.05) reduction.

Semen analysis on day 60 showed a significant increase in sperm count in high dose propolis administered rats, while sildenafil and paroxetine untreated rats caused significant (p<0.05) sperm count reduction. Propolis also caused a sustained sperm motility, while sildenafil and paroxetine untreated rats revealed significant (p<0.05) reduction in sperm motility. The total
sperm abnormality was also significantly (p<0.05) decreased by propolis while sildenafil sustained the total sperm abnormality. However, paroxetine untreated rats led to significant (p<0.05) increase in total sperm abnormality.

The LH assay results showed that there was significant (p<0.05) increase in propolis-sildenafil combination treated rats and significant (p<0.05) reduction in other propolis administered, paroxetine untreated rats, and sildenafil treated rats when compared to control (group I). This shows that propolis-sildenafil combination has a protective role on LH being able to maintain the plasma level of LH especially at high dose. An indication that there is possibility of an interaction between propolis and Leydig cells. The FSH levels of non-induced propolis-tested rats (group II) and propolis-sildenafil combination treated rats (group VIII) exhibited significant (p<0.05) increase when compared to control group. While there was significant (p<0.05) reduction in paroxetine-induced untreated rats, all other treated groups (IV-VII) were not statistically significant (p>0.05) as compared to control. Remarkable reductions in testosterone level that were significant (p<0.05) were noticed in paroxetine-induced untreated rats (group III) and 200mg/kg propolis-treated rats (group VII). However, all other propolis-administered rats (groups II, V, VI, VIII) and sildenafil-treated rats (group IV) were not significantly (p>0.05) different from the control.

The histology generally showed I, II, VI and VII with organized seminiferous tubules and a normal spermatogenesis cycle with even distribution of every cell stage throughout the tubules and multiple germ cells, with aggregations of spermatogenic cells around the lumen with populated spermatozoa. However, III and IV revealed disorganized seminiferous tubules with large interstitial spaces.
**Conclusion:** Propolis has a key role to play in reproductive functions in enhancing sexual indices if given at different appropriate doses and timing in post-SSRIs sexual dysfunction, through modulation of nitric oxide and reproductive hormones.
الخلفية: وجدت العديد من الدراسات وجود صمة بين النباتات الطبية لإنتاج إجراءات فسيولوجية محددة في أنظمة الجسم. يتم عرض العديد من المنتجات كمعزز جنسي طبيعي، ولكن لم يتم توثيق أي تجارب سريرية أو دراسات علمية لدعم فعاليتها لعلاج الضعف الجنسي بدافع الفضول، دنج (PL) وهو خليط يجمعه نحل العسل من أجزاء من النباتات، البراعم، الإفرازات، براعم الأشجار، تدفقات النسغ يجلب بالفعل الكثير من الاهتمامات البحثية. خاصة الآن يجري استهلاكا محليا لتعزيز الجنسي. تشير التقديرات إلى أن ما يصل إلى 50 - 80% من المرضى الذين يعانون من ضعف جنسي ما بعد (PSSD) يتناولون من SSRI (PSSD) واحدة أو أخرى. هدفت الدراسة الحالية إلى استكشاف وتقييم الأنشطة الطبية للدنج باستخدام نموذج حيواني بهدف تبرير الادعاء العرقي من استخدامه الأصلي ليكون مفيدًا في تحسين علاج الاختلالات الجنسية المختلفة.

الطريقة: تم استخدام جرذان ويستر ذات القيمة الجنسية العالية بين 140 جرام و 190 جرام، وعدد مماثل من الإناث غير البكر جنسياً (120 جم-130 جرام) في هذه الدراسة. كان إحداث العجز الجنسي في ذكور الجرذان عن طريق إعطاء باروكستين، وتأكد من خلال تعريضهم جنسياً للأنثى الأخرى. تم تقسيمهم عشوائياً إلى ثمان مجموعات من ستة فئران كل (N = 6)، وكانت إما تركت مع السيارة وحدها أو السيدينافيل المدارة، أو تدبير ضمور متدرج من PL أو سيلدنافيل بالاشتراك والدنج شفويا خلال فترتين مختلفتين من 7 أيام و 60 يومًا. في نهاية هذه
الآيام، تم اختبار السلوك التنظيمي بين عشية وضحاها لإجراء تقييم بصري للمؤشرات الجنسية (MF, IF, EF, ML, IL, EL و PEI) والتي تم بعدا التضحية بالحيوانات من أجل جمع السائل المنوي لتحليل السوائل المنوية، واختبار الخصائص لتقييم النسيجي. بالإضافة إلى ذلك، تم جمع عينات الدم من الحيوانات التي تدار لمدة 60 يومًا للتحليل البيوكيميائي من LH، FSH، التيستوستيرون وأكسيد النيتروجين.

النتائج: كشف نشاط الاستنساخ في اليوم السابع أن جرعة عالية من البروبوليس وفياغرا حافظت على كل ترددات النوبات بشكل عام فيما عدا EF التي كانت معنوية (P<0.05) مخفضة بواسطة سترات السيلدينافيل، بينما انخفضت كل هذه بشكل ملحوظ (P<0.05) في الجرذان غير المعالجة بالباروكستين. على نفس المنوال، دنج الجرعة العالية والاستنساخية باستثناء IL التي زادت بشكل ملحوظ، في حين كانت كلها بشكل ملحوظ (P<0.05) زيادة في الجرذان غير المعالجة paroxetine. الليمي أيضاً مدعوماً بجرعة عالية من دنج وسيلدينافيل.

أظهر نشاط الاستنساخ في اليوم 60 أن دنج استمر، وزاد بشكل كبير (P<0.05)جميع ترددات الدعامة. كما عانى فياغرا جميع ترددات الاستنساخ باستثناء IF التي كانت معنوية (P<0.05) مع تأخر، وبشكل ملحوظ (P<0.05) مخفضة. ومع ذلك، فياغرا، على الصعيد العالمي، زيادة كبيرة في جميع حالات الطوارئ الاستنساخ. كما تم الحفاظ على من قبل دنج جرعة عالية وسيترات السيلدينافيل.
كشف تحليل السائل المنوي في اليوم السابع عن زيادة معنوية (P<0.05) في عدد الحيوانات المنوية في إعطاء البروبوليس بجرعات عالية، في حين كان هناك انخفاض معنوي (P<0.05) في الفئران غير المعالجة والفايروكسيد. كما تم الحفاظ على حركة الحيوانات المنوية من خلال دنج جرعة عالية، في حين كانت كبيرة والباروكسيتين غير المعالجة.

تم تخفيض الشذوذ الكلي للحيوانات المنوية أيضا عن طريق دنج جرعة عالية. في حين أن فياغرا حافظت على الشذوذ الكلي، تسبب الحيوانات غير المعالجة بالباروكسيتين في خفض كبير (P<0.05).

أظهر تحليل السائل المنوي في اليوم 60 زيادة ملموسة في عدد الحيوانات المنوية في الجرذان التي تدار بجرعات عالية من البروبوليس، في حين أن الجرذان غير المعالجة والفازورين غير المعالجة تسببت في تخفيض عدد الحيوانات المنوية (P<0.05) دنج. تسبب أيضا في حركة الحيوانات المنوية المعالجة والفايروكسيد paroxetine المعالجة، انخفض أيضا في جمالي شذوذ الحيوانات المنوية بشكل كبير بينما فياغرا حافظت على جمالي شذوذ الحيوانات المنوية. ومع ذلك، أدت الجرذان غير المعالجة بالباروكسيتين إلى زيادة معنوية (0.05<P) في إجمالي شذوذ الحيوانات المنوية. دنج في الجرعات العالية وفياغرا كانت قادرة على الحفاظ على مستويات FSH البلازما من هورمون النمو والتستوستيرون في حين كان مستوى البلازما من بشكل ملموسة (P<0.05) ومع ذلك، فإن الحيوانات غير المعالجة بالباروكسيتين على مستوى العالم خفضت مستويات البلازما من جميع الهرمونات بشكل ملموسة (P<0.05) كان مستوى البلازما من أكسيد النيتروجين أيضا بشكل ملموسة.
 región (P<0.05) زيادة من دنج جرعة عالية وفياغرا ، في حين تم تخفيض الحيوانات غير المعالجة الباروكستين بشكل كبير (P<0.05)

أظهر علم الأنسجة عموما I، II، III و VII مع الأنبوب المنوية المنظمة ودورة حياة الحيوانات المنوية العادية مع توزيع منتظم لكل مرحلة خلية في جميع أنحاء النببيات والخلايا الجرثومية المتعددة وتجمعات الخلايا المنوية حول التجويف مع الحيوانات المنوية المأهولة. ومع ذلك، كشفت الثالث والرابع النببيات المنوية غير المنظم مع مساحات بينية كبرى.

الاستنتاج: للدوبول دور رئيسي يلعبه في علم وظائف الأعضاء الإنجابية إذا تم إعطاؤه عند تناول الجرعات المناسبة المناسبة والتوقيت في الخلل الوظيفي التالي لـ SSRI من خلال تعديل أكسيد النيتريك والهرمونات التناسلية.
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Chapter One: Introduction and Literature Review

1.1. Introduction

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