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ABSTRACT:
Propolis is a resinous product collected by honey bees for construction of hives. It is locally used as remedy for several ailments including various degrees of sexual dysfunctions. This study investigated the protective property of propolis in improving sperm quality in paroxetine-induced sexually impaired male Wistar rats. Forty-two male rats were divided into 7 groups of 6 rats each. Groups I (normal saline), II (administered paroxetine only for two weeks), III (Sildenafil), IV (low dose propolis), V (moderate dose propolis), VI (high dose propolis), and group VII (propolis+sildenafil). There was significant ($p< 0.05$) increase in sperm count in group VI, no significant ($p>0.05$) change in sperm count of group VII, but significant decrease in the other groups compared to control. There was significant ($p< 0.05$) reduction in sperm motility of rats in groups II, III, and IV, compared to significant ($p< 0.05$) increase in sperm motility of rats in groups VI and VII, but there was no significant ($p>0.05$) change in group V. The total sperm abnormality in groups II, III and IV showed significant ($p<0.05$) increase, while there was significant ($p<0.05$) reduction in sperm abnormality of groups VI and VII compared to control, and no significant ($p>0.05$) difference seen in group V. The plasma testosterone levels were significantly ($p<0.05$) reduced in groups II, III and IV, compared to significant ($p< 0.05$) increase in plasma testosterone level in groups VI and VII, but no significant ($p> 0.05$) difference in plasma testosterone in group V. The results indicate that high doses of propolis caused significant increase in plasma testosterone, and there was improvement in sperm count and motility as was obtained by the analysis of seminal fluid (SFA).

Keywords: Propolis, Sperm, Infertility, Sildenafil citrate, Paroxetine

INTRODUCTION:

Propolis is a natural brownish-green resinous substance produced by honey bees. Bees use propolis (PL) in making protective shield and filling of cracks of their hives, and also to polish the cells of the honeycomb [1]. PL is considered as one of the most promising natural products with capacity for treatment and prevention of myriads of health conditions [2]. PL is reported to have several pharmacological effects; as hepatoprotective, anti-inflammatory and antioxidant properties [3,4]. Sexual activity involves several aspects that are associated with complex interactions between neuroendocrine and vascular systems including a variety of structures that are instrumental to sexual excitement, intercourse and sexual pleasure [5,6]. Sexual dysfunction is a common side effect of some psychoactive medications as well as a number of other frequently prescribed drugs. Considerable attention has recently been focused on reproductive side effects of antidepressants, which are often taken for long duration; perhaps because they are usually under-reported by sufferers due to associated stigma [7]. Paroxetine is one of the Selective Serotonin Re-uptake Inhibitors (SSRIs) that are frequently prescribed for patients suffering from depression, anxiety or obsessive compulsive disorder. Common reproductive side effect of psychoactive drugs is a constellation of several entities described as sexual dysfunction which includes erectile dysfunction, decreased libido, delayed orgasm, and aspermia [8]. Sildenafil citrate is an oral medication considered to be first line therapy for erectile dysfunction. It is a specific Phosphodiesterase type-5 (PDE5) inhibitor that promotes penile erection by blocking the activity of PDE5 which causes cyclic-Guanosine Monophosphate (cGMP) to accumulate in the corpus cavernosum [9,10]. With the widespread increase in the use of antidepressants following increase in the prevalence of new cases of depression [11], and the consequent increase in antidepressant-induced infertility in males, especially due to affectations of semen parameters, it is pertinent to find appropriate medication that is natural, affordable, safe and easily available to manage this condition clinically. In view of the above, this this study was aimed at investigating the effects of propolis on the quality of semen in paroxetine-induced sexually impaired male Wistar rats.

METHODOLOGY:

Sexually potent male rats weighing between 140–190g, and equal number of sexually unexposed female rats weighing between 120-130g purchased from National Center for Research, Khartoum, Sudan were used for this study. They were housed separately and maintained in the animal house of the Faculty of Veterinary Medicine, University of Khartoum, Shambat, Sudan. They were kept in standard plastic cages containing wood chips (sawdust) bedding, with good ventilation, free access to

standard rat pellet feeds (National Center for Research) and water *ad libitum*. The animals were subjected to artificial day and night cycle of 12 hours by 12 hours light, under optimum temperature of 25-30^{o}C. The animals were acclamatised for two weeks prior to commencement of the experiment. The experimental protocols which involved invasive and non-invasive procedures were approved by the Animal Research Ethical Committee of the International University of Africa, Khartoum, Sudan, and were conducted in accordance with internationally accepted principle for laboratory animal use and care.

Propolis extract was a product of Organic Bee Farms Sheridan, Illinois (IL) Unites Stated of America (USA). It is commercially prepared as 1000mg per capsule and reconstituted by dissolving in 10ml of normal saline and delivered as 100mg/ml.

Paroxetine hydrochloride was a product of Pharaonia Pharmaceuticals, New Borg El-Arab City, Egypt. It was purchased at Hashmik Pharmacy in Khartoum Sudan. Ten milligram of the drug was dissolved in 10ml normal saline and delivered as 1mg/ml.

Sildenafil citrate (Viagra) was a product manufactured by Cadila Health Care Limited, Sharkhe-Bavla Ahmedabad India. It was purchased at the pharmacy outlet of the International University of Africa, Khartoum, Sudan. Twenty five milligram of the tablet was reconstituted in 10ml of normal saline and delivered as 2.5mg/ml.

Forty-two male rats were randomly distributed into seven groups containing six rats each; following administration of 10mg/kg of paroxetine for two weeks for induction of sexual dysfunction of groups II-VII as evident by loss of sexual behaviours, such as, mount and intromission frequencies [7]. The various treatments administered to the rats in the six experimental and control groups are presented in Table 1. The rats in Group I served as the general control and was maintained on 0.9% normal saline. Rats in Group II were given 10mg/kg of paroxetine and left untreated. Rats in Group III were given 25mg/kg of sildenafil in addition to 10mg/kg paroxetine. Rats in Group IV were given 50mg/kg of propolis (low dose) in addition to 10mg/kg paroxetine. Rats in Group V were given 100mg/kg of propolis (moderate dose) in addition to 10mg/kg of paroxetine. Rats in Group VI were given 200mg/kg of propolis (high dose) in addition to 10mg/kg of paroxetine. Rats in Group VII were given combination of 200mg/kg of Propolis and 25mg/kg of sildenafil in addition to 10mg/kg of paroxetine. The duration of the treatment was 60 days.

Table 1: Treatment administered to rats in the control and experimental groups

Groups of rats	Normal Saline solution	Paroxetine (PT) (mg/kg)	Sildenafil (SF) (mg/kg)	Propolis (PL) (mg/kg)
I	0.9%	0	0	0
II	0	10.0	0	0
III	0	10.0	25.0	0
IV	0	10.0	0	50.0
V	0	10.0	0	100.0
VI	0	10.0	0	200.0
VII	0	10.0	25.0	200.0

At the end of the experimental period, rats were sacrificed under ketamine anesthesia. An abdominal midline incision was made and extended to the chest to expose the heart. The apex of the heart was punctured with a needle and syringe. Blood sample was collected and poured into appropriately labeled heparinized blood collection tube. The tubes were centrifuged at 3000 rpm for 15 minutes to separate the plasma, which was then separated and stored appropriately till required for analysis. The quantitative determination of total testosterone concentration in plasma was done using Microplate Enzyme- linked Immunoassay (EIA) using Monobind assay kit (Lake Forest, USA) according to the manufacturer's instructions. Semen sample was obtained from the caudal region of the epididymis of each rat by carefully milking down the vas deferens. The sample was milked directly into a petrish dish already filled with diluent prepared from non-fatty milk powder (11%, w/v) and distilled water heated to 95°C for 10 min. After cooling to room temperature, penicillin (64.2mg) and streptomycin (100mg) were added at 37°C. Following this, the semen and diluent were gently mixed together with the tip of a pipette (sucking and release) being careful to avoid trauma to the spermatozoa. About 15µL of the diluted semen was then pipetted on to a glass microscope slide and cover slip placed on top. It was then observed under light microscope at ×40 magnification. Sperm quality was determined through assessment of the following parameters: sperm concentration, motility, and morphology [12, 13].

Sperm concentration was analyzed using the haemocytometer method [14]. The diluted semen sample was put into the counting chamber, and the number of spermatozoa was counted using a haemocytometer with improved doubles Neubauer ruling under a light microscope. The number of spermatozoa in five squares was counted. The mean was multiplied by 10^6 in order to obtain the sperm count. The sperm concentration was expressed as $\times 10^6$ ml^{-1}.

Sperm motility was analyzed and averaged by counting the motile and non-motile spermatozoa under a light microscope and expressed as the percent motility [14]. The

motility was determined by eye-estimation of the proportion of spermatozoa moving progressively straight forward at higher magnification (x40) and expressed as percentage. With this assessment, the spermatozoa were classified into three categories according to their motility: progressive, in situ and immobile. The spermatozoa with progressive motility are those with lineal forward movements; in situ motility refers to those with circular or local movements, and immobile sperm are spermatozoa without movements [15]. The motility was observed using the ×40 microscope objective lens. The number obtained in each category was expressed as a percentage.

Sperm total abnormality was assessed by adding two drops of warm eosin–nigrosin stain [14] to the semen (10µl) on a pre-warmed slide. A uniform smear was then made and air-dried. The stained slide was immediately examined under an oil immersion lens. For each rat, 200 spermatozoa were examined randomly for abnormalities in the head and/or the tail in different fields, and the percentage of total abnormalities was determined [16]. The normal spermatozoa, with a sickle-shaped head and large flagellae versus the abnormal ones with double head and fragmented or zigzag flagellae were distinguished.

Analysis of data was done using SPSS version 20. Results were presented as mean ± standard error of mean (SEM). Differences between groups were analysed by one way analysis of variance (ANOVA) followed by LSD post-hoc test. In all statistical analyses, differences were considered significant at $p < 0.05$.

RESULTS:

The results obtained are presented in Table 2. The 10mg/kg paroxetine significantly reduces the sperm count of rats in group II compared to the control rats in group I. There was also significant ($p<0.05$) reduction in sperm count of rats in groups III, IV, and V. However, there was significant ($p< 0.05$) increase in sperm count of rats in group VI, but no significant ($p> 0.05$) change in the sperm count of rats in group VII compared to the rats in the control group.

The 10.0mg/kg paroxetine also significantly ($p< 0.05$) reduced the sperm motility of rats in group II compared to the control. The sperm motility of rats in groups III and IV were also significantly reduced compared to the rats in the control group and in group II. The sperm motility of rats in groups VI and VII was increased significantly, but the increase observed in VI was more remarkable than in group VII. However, there was no significant ($p>0.05$) change in sperm motility of rats in group V.

The total sperm abnormality seen in groups II, III and IV showed significant ($p<0.05$) increase, though the increase in sperm abnormality seen

in group II was more than that seen in groups III and IV. There was also significant ($p<0.05$) reduction in sperm abnormality of rats in groups VI and VII compared to control, however, the reduction in group VI was more remarkable than that of group VII. There was no significant ($p>0.05$) difference in sperm abnormality observed in group V compared to the control group.

The plasma testosterone level in group II was significantly ($p<0.05$) lower compared to the control and other groups. Significant ($p< 0.05$) increase was observed in plasma testosterone level of rats in groups VI and VII. However, there was no significant ($p> 0.05$) difference in plasma testosterone of rats in group V compared to the control.

Table 2: Effect of propolis on SSRIs-induced sexual dysfunction on quality of semen and testosterone

Parameter	Group I	Group II	Group III	Group IV	Group V	Group VI	Group VII
Sperm count ($x10^6$)/ml	236.17± 1.87	46.50± 1.02*	119.50± 1.23*^{b}	117.50± 1.84*^{b}	120.33± 0.84*^{b}	253.83± 0.67*^{b}	232.67± 1.59^b
Sperm motility %	78.67± 0.88	28.83± 2.89*	51.67± 1.05*^{b}	62.83± 1.20*^{b}	79.83± 0.60^b	88.83± 1.20*^{b}	87.83± 2.14*^{b}
Total abnormality %	29.00± 1.63	79.67± 3.96*	52.83± 0.95*^{b}	44.00± 1.21*^{b}	32.67± 1.12^b	18.33± 1.61*^{b}	21.00± .53*^{b}
Testosterone (nmol/l)	4.07± 0.14	1.53± 0.05*	3.09± 0.19*^{b}	3.51± 0.12*^{b}	4.18± 0.13^b	4.93± 0.03*^{b}	4.98± 0.07*^{b}

*$p<0.05$ when compared to control (group I); $^b p<0.05$ when compared to paroxetine treated only (group II)

DISCUSSION:

The result obtained for rats in group VI indicates that the 200mg/kg propolis tends to have reversed the suppressive effect of the 10mg/kg paroxetine and, at the same time, enhances sperm production in the rats. A full reversal to normalcy was obtained in rats in group VII that received the combined dose of 25mg/kg sildenafil and 200mg/kg propolis. The reduction of the sperm count in group II (untreated) was highly significant, perhaps because paroxetine overwhelmingly destroyed its testicular cells than that of other treated animals. The increase sperm count above was in tandem with the findings of Ayinde et al. in 2019 [7]. They observed significant elevation in plasma level of FSH in rats that received 10mg/kg paroxetine and high doses of propolis which could have been responsible for stimulation of Sertoli cells that heralded increase spermatogenesis. In the same vein, Al-sayed *et al.,*[17] also reported that propolis aided improvement of male rat fertility affected by aluminum chloride cytotoxicity which also supports the outcome of this study though with paroxetine toxicity. The present study is also in

concordance with the work of Capucho *et al*., [18] who observed that propolis increased sperm production and the epithelial height of the initial segment of the epididymis. The sperm motility in rats receiving high dose propolis (group VI) and propolis-sildenafil (group VII) was elevated, and even more significant in VI than VII. This indicates that PL can reverse the negative effect of paroxetine, and has the propensity to preserve the movement ability of spermatozoa which is a critical factor in the fertilizing capacity of spermatozoa in spite of the destructive effect of paroxetine.

This could be as a result of the antioxidant property of flavonoids in propolis that could have possibly reversed paroxetine toxicity in the energy system of the germ cells of the testis [19]. This result supports the in vitro study by Miroslava et al. in 2014 [20]. They reported that PL can improve sperm motility. However, the sperm motility of rats in groups III and IV was substantially reduced, except in group V where the effect was uneventful.

The total sperm abnormality counted was reduced appreciably in high dose propolis (group VI), and propolis-sildenafil combination (group VII) treated rats. Because PL is known to be a scavenger of free radicals [21], it has the tendency to prevent oxidation of cell membrane lipids. The low total sperm abnormality seen in this study supports the findings of Fischer et al. in 2007 [22]. They found out that PL can preserve cellular immune response by increasing mRNA for interferon-γ and onward activation of cytokines, thus accelerating anti-inflammatory processes. In contrary, abnormality in group II, untreated paroxetine administered rats was globally increased. This is not surprising because of the destructive side effect of paroxetine that could have affected the germinal cells of the testis.

The total structural abnormality of sperm cells was increasingly more observed in paroxetine untreated, sildenafil alone, and low dose propolis treated rats intervention. This can be arrogated to the residual destructive effect of paroxetine concentrated in the testicular tissues that has not been completely metabolized in the rats. It was not surprising that the group given sildenafil intervention alone could not remedy the structural abnormality observed in sperm cells caused by destructive effect of paroxetine. This is because sildenafil is only known to be erectile enhancing drugs and not an antidote for structural abnormality in sperm cells. The sperm abnormality of group V treated rats was also not affected over the period of the experiment, this showed that moderate dose of propolis also confers some level of protections on sperm cells. At a high dose of propolis (200mg/kg), plasma level of testosterone increased. This could have been responsible for the elevation in sperm count due to alteration in the hypothalamo-pituitary-testicular axis. The result of this study supports the findings of Mokhtar et al. in 2010 [23]. They

concluded that PL increased testosterone level and improved the quality of semen. Doses of propolis, and sildenafil administration, groups II, III and IV showed significant decrease in, plasma level of testosterone, while groups VI and VII reflected significant increase except in group V that had uneventful plasma testosterone level.

CONCLUSION:

From the result of the present study propolis caused a dose-dependent alteration in plasma testosterone level. The high dose propolis caused significant increase in the level of plasma testosterone which led to improvement in sperm count and motility as seen in the analysis of the seminal fluid.

Recommendation

In the present study the results obtained show that propolis can cause alterations in the fertility parameters involved in the reproductive system of male rats. However, more study is needed to fully understand some other physiological mechanisms involved, and to further explore any other benefits inherent in propolis on the reproductive system of male rats.

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