

EVALUATION OF THE EFFECT OF *WITHANIA SOMNIFERA* EXTRACT ON SEX HORMONE AND GONADOTROPIN LEVELS IN ADDICTED MALE RATS THAT INDUCED BY MORPHINE

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ABSTRACT : The consumption of opioid has been widely increasing across with thousands of deaths and tens of millions of addict people. One of the significant unwanted effects of opioids is sexual dysfunction since opioids can reduce fertility and sex hormones. *Withania somnifera* (WS) is a common herb which is used in India to enhance sexual activity. The aim of the study is to evaluate the effect of WS in morphine-addicted male rats on gonadotropins and sex hormones. Sixty male rats were randomly divided into four groups: control group, addicted group, WS-treated control group, and WS-treated addicted group. In all groups, the plasma level gonadotropin and sex hormone were determined. We found that the use of morphine reduced the majority of gonadotropin and sex hormone levels, except for follicle-stimulating hormone (FSH). However, WS induced a substantial elevation in hormones. In conclusion, morphine administration appears to reduce sex hormone and gonadotropin levels, especially estrogen, LH and testosterone levels while *Withania somnifera* extract counteracted morphine-induced low levels of hormones.

Key words : Estrogen, *Withania somnifera*, morphine, testosterone, gonadotropins.

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INTRODUCTION

The recent years have witnessed a widely spread use of opioids such as fentanyl and oxycodone. Such use is accompanied by the appearance of the significant unwanted effect which is the addiction (Moussawi *et al*, 2020). Briefly, opioids are pain killers belong to the naturally present "opiate" family. Opioids are strong analgesics which are either exogenous (obtained from plants or may be semisynthetic) or endogenous (found in animals and humans). They are centrally acting to alleviate pain and discomfort. They are mainly used in the management of acute severe pain such as pain associated with myocardial infarction and during labor with possible use in chronic pain (Ballantyne and Mao, 2003; Cohen *et al*, 2017; Cornwell *et al*, 2020). However, opioids cause use-limiting side effects such as nausea, vomiting (Kanemasa *et al*, 2020), constipation, lightheadedness, dizziness and drowsiness (Hachon *et al*, 2017), sweating, dry mouth, ventilatory failure, sleep apnea (Dalesio *et al*, 2020), mental/mood changes such

as restlessness, hallucinations, confusion (Sivanesan *et al*, 2016), trouble urinating, changes in vision, slow / fast heartbeat, extreme stomach/abdominal pain (Tessier *et al*, 2019). In addition, signs of disturbed adrenal function such as loss of appetite, unusual tiredness, weight loss (Pincus *et al*, 2017). In human beings as well as in animals, in males as well as females, morphine can negatively affect sexual hormones and fertility (Siddiqui *et al*, 1997). For example, morphine caused reduced libido, increased rates of stillbirth, and genetic defects (Zagon *et al*, 1977). In addition, it may cause testosterone deficiency in men who also have sexual dysfunction, low bone mass, depressive symptoms, and reduced quality of life (Basaria *et al*, 2015).

Not only sexual and hormonal disorders, but also disorders of human behavior may be caused by chronic use of morphine (Gu *et al*, 2008; De Graaf *et al*, 2013). No insignificant treatment has been approved to counteract these conditions. Nevertheless, during the past three decades, the use of herbal medicinal products and

supplements has increased enormously, with no less than 80% of individuals worldwide dependent on them for some aspect of primary health care (Ekor, 2014). Therefore, researchers are trying to identify new medicines among herbal medicines, which have less complications.

Withania somnifera is a plant that is commonly known as Ashwagandha. It is considered as the “ginseng of India” (Mishra *et al*, 2000). It is used to treat a wide range of medical conditions owing to its chemical composition including tannis, flavonoids, antioxidants, and phenolic compounds with a wide variety of activities (Chaudhuri *et al*, 2012). The pharmacological benefit of *Withania somnifera* is confirmed by its use in various disorders of the central nervous system, particularly its indication in epilepsy, stress and neurodegenerative diseases like alzheimer’s and Parkinson’s disorders, cerebral ischemia, tardive dyskinesia and even in drug addiction treatment (Kulkarni *et al*, 2008). In fact, the medicinal properties may be related to secondary metabolites such as withasteroids-withanolides and alkaloids (Konar *et al*, 2020). Among different functional groups, some of anolides may have specific therapeutic significance (Tiwari *et al*, 2020).

This research aimed to investigate the influence of *Withania somnifera* on gonadotropins and sex hormones and sexual disorders in opioid-addicted animals.

MATERIALS AND METHODS

Experimental animals (change)

In our study, 60 adult male wistar rats weighing from 200 to 250 g were obtained from the University of Baghdad, Faculty of Science. Under uniform house conditions on a daily temperature of $24 \pm 2^\circ\text{C}$ with free admission to food and tap water, the animals were held in cages and 12 hours of light-dark cycling was maintained.

Experimental design

Rats were randomly divided into four groups: group 1 (control), group 2 (WS-treated), group 3 (morphine addict) and morphine addict/WS-treated. Water-soluble morphine was administered for the induction of morphine addiction in both groups 3 and 4 for 21 days, and WS plant-mixed pelleted food for group 2 and 4. Then animals were held in animal houses for week.

Induction of morphine addiction

Morphine solution at doses of 0.1, 0.2 and 0.3 mg / ml was administered to rats; each dose was administered for 48 hours and then 4 mg/ml was administered for the remaining 15 days, because of the unpalatable taste of morphine solution and to prevent morphine bitterness, 3%

sucrose was added to the solution. Intraperitoneal injection of 2 mg / kg of naloxone and observation of side effects had confirmed addiction (Yadegari *et al*, 2011).

Preparation of *Withania somnifera*

WS roots were cut into small pieces (1~2 cm), dried at room temperature for 24 h (Kulkarni and Ninan, 1997) and mixed with pelleted rat food at a ratio 6.25/100 (62.5 g of WS with 1000 g of rat chow pellet) followed by grinding of the mixture. New food pellets were produced from the final mixture using a pellet-forming device. For 21 days, WS mixed pelleted food was obtained by the treated groups (2 and 4) (Kiasalari *et al*, 2011). Animals were received 0.3 ± 0.01 g / kg WS daily, given the daily intake of pellet weight (5 /kg/d) in rats and 6.25% WS in new pellets.

Blood sampling

Blood (3-5 ml) was taken by cardiac puncture and the blood plasma was isolated from by centrifugation at 2000 rpm for 15 minutes, which was stored for hormone analysis in a freezer at -70°C .

Plasma analysis

Using ELISA kits (Labsystems Ltd., Finland) in addition to kits developed by Monobind Inc., USA, the plasma levels of gonadotropins and sex hormones were measured. The measurements were performed at Shahid Mostafa Khomeini Hospital’s central laboratory.

Statistical analysis

Data were presented as mean \pm SEM and SPSS software was used to analyze data. One-way analysis of variance (ANOVA) was used to test statistical differences, followed by the post-hoc Tukey test. Those data with $P < 0.05$ were considered significantly different.

RESULTS

As shown in Table 1, morphine addiction resulted in a significantly decreased level of estrogen relative to the control group, and also caused a decrease in the level of progesterone but at a lower level effect, while WS treatment produced a significant increase in level of estrogen in compartment control group ($P < 0.05$). There appears to be no major effect on the estrogen level of addicted male rats, although the levels of progesterone in the addicted group have decreased significantly lower to the control group and the WS-treated group, in additionally progesterone levels has increased significantly. In addition, the table shows the level of testosterone was decreased by administration of morphine to 0.113 ng/mL relative to the control group (0.348 ng/ml, $P < 0.05$). WS-treated morphine addiction, on the other hand, significantly increases ($P < 0.05$)

Table 1 : The effect of morphine and *Withania somnifera* on sex hormone in male rats.

| Groups | Estrogen(pg/ml) | Progesterone level | Testosterone level | FSH | LH levels |
|----------------------------------|-----------------|--------------------|--------------------|--------------|----------------|
| Control group | 52.57 ± 3.1a | 52.24 ± 3.2a | 0.348± 0.2 a | 0.287± 0.1 a | 0.191± 0.05 ab |
| WS treatment only | 88.92 ± 2.7b | 68.30 ± 6.1a | 0.359 ± 0.3 a | 0.425± 0.3 b | 0.213± 0.012b |
| Morphine group | 32.21± 1.2c | 28.76 ± 1.1b | 0.113± 0.15b | 0.35± 0.1 c | 0.0132± 0.002c |
| Morphine group plus WS treatment | 50.43 ± 4.1a | 58.48± 5.1a | 0.289± 0.1 a | 0.33± 0.3 c | 0.176± 0.05a |

abc with different superscript across the rows indicate significant differences ($P < 0.05$).

testosterone levels (0.289 ng/mL).

Finally, the results show that FSH had a statistically significant increase ($P < 0.05$) compared to the control group in the WS-treated control group only, while data on levels of LH in all groups showed that there was a significant decrease in levels of LH compared to control group in the addicted male rats. However, the WS-treated morphine group showed significant increases in the levels of LH in addicted rats.

DISCUSSION

The main objective of this project was evaluated the effect of morphine an addiction and treated by *Withania somnifera* on mammalian sex hormone. Since, our data suggest that administration of *Withania somnifera* to addicted male rats by morphine causes increased in mammalian sex hormone. Which the result showed morphine addiction cause many significant adverse, since causes decreased in LH, testosterone, and estrogen secretion but not levels of FSH and progesterone related to the control group as agreed post studies (Jalili *et al*, 2016), which showed long time used of morphine causes reduced testosterone secretion and reduced number of sperms consequently as well as decreased in libido (Lynch *et al*, 2020). Additionally, heroin addiction that effected on pituitary testicular function which detected that causes inhibitory factor on LH secretion and sperm parameters that as express in studied (Nazmara *et al*, 2019) and reduce gonadotropins by modifying the sex hormone-hypothalamic feedback (Medina *et al*, 2020). The result showed morphine decreased the estrogen level significantly, while compared with WS-treated control group which estrogen level increased significantly. In contrast, the levels of hormone maintained its concentration in the WS-treated addicted group. Result finding conformity with another result studies (Durg *et al*, 2018) which showed increased in the estrogen and progesterone level after administration of WS.

In this study, the effect of WS use in the morphine-addicted group treated was insignificantly compared with that of the control group in which morphine did not substantially change progesterone levels. Our results also showed that the reductive effect of morphine on LH and testosterone was antagonized by dietary intake of the

WS root. Some studies shown that some effects of morphine's key are counteracted with WS root methanol extract administration (Ruiu *et al*, 2013).

It seems that WS counteracts the morphine-induced inhibition of pituitary gland. Having this effect, WS can enhance the release of LH from the pituitary and subsequently enhance the testosterone level in plasma. Elevated concentration of testosterone in the plasma leads to a secondary increase in estrogen level. Morphine addiction has no major impact on the level of FSH in addicted male rats, so WS also did not affect the level of FSH relative to the WS-treated control group where the levels of LH and testosterone do not show significant changes whereas the levels of FSH and estrogen tend to be highly increased in their levels. Therefore, depending on the animals' treatment, WS tends to have affected sex hormones and gonadotropins differently.

In agreement with our results, several studies have reported that testosterone levels are less than required in addict male rats. In addition, other studies have demonstrated a positive effect of WS on the level of LH in adult male rats (Abdol-Magid *et al*, 2001). Another study reported that WS disrupt libido and male sexual behavior in rats more or less in a similar manner as shown *W. somniferous* roots possess GABA mimetic activity in adult rats (Lynch *et al*, 2020).

CONCLUSION

Generally, the administration of morphine appears to decrease levels of sex hormone and gonadotropin especially levels of LH, testosterone and estrogen. However, the administration of WS extract to the addicted rats causes a significant increase in testosterone and LH levels which can be used in treated men infertility.

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