



Evaluation the Protective Effect of *Withania somnifera* Extract on the Level of Sex Hormone in Morphine Addicted Female Rats

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Abstract

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BACKGROUND: Morphine is one of the most types of phenanthrene alkaloid opioid used to soothe the acute and chronic pain through narcotic and analgesic medical employment. Increasingly, constantly used of opioid in the public and medication practical important knowledge improve that. Morphine shows that pernicious has effects on numerous tissue for instance ovary, liver, and lung morphine side effects instruct for existence of oxidative role due to generation of reactive oxygen species in the affected tissue. The past decades researchers proved that natural substance provides protective role against toxic effect. Thus, *Withania somnifera* consider as antioxidant substance provides protective versus the toxic substance as morphine.

AIM: The present study was aimed to evaluate the protective role of *W. somnifera* extract on the level of sex hormone in morphine addicted female rats.

METHODS: The experiment use of 40 adults female Wistar rats, the body weight was ranged between 200 and 250 g. In the present study, the animals divided in the four equal groups as described followed; first group indicated as control group which that received placebo only, while in the second group indicate as induction group) and third (treatment group) group which both groups received water-soluble morphine for the addictive creation of morphine properties. Moreover, the third group received 6.25% *W. somnifera* extract in new pellets for 21 days. Finally, the fourth group (*Withania S.* group) received 6.25% *W. somnifera* extract in new pellets for 21 day only.

RESULTS: The result showed significant decrease of LH in the morphine groups after 21 days of experimental compared with the control and treatment groups, in addition, exhibited that not significant differences among control and treatment groups that treated by 6.25% *W. somnifera* extract in new pellets for 21 days. Interestingly, that *Withania S.* group exhibited improvement hormonal regulation.

CONCLUSION: In conclusion, the present study confirmed a truth evidence of a protective roles of *W. somnifera* against the morphine addiction in female rats and used as promoter effect.

Introduction

The opioid drugs recently are clinically practice position used as adjuvant analgesics for cancer pain, after an operation or a serious injury and heart attack [1], [2]. Morphine is one of the most types of phenanthrene alkaloid opioid used to alleviate acute and chronic pain [3]. As well as, scientists suggested that administration of morphine in low dose can act as neuroprotective role in animal models of Parkinson's disease through decrease of sarcoplasmic reticulum stress, additionally to a decrement of oxidative tissue stresses simultaneously [4]. However, long-term administration of morphine leads to tolerance to its analgesic effect and could even cause drug dependence as well as, that morphine has adverse effect reproductive system [5]. Scientists with tensive continue research to identify the molecular pathways regarding morphine hazardous effects on tissues [6], [7]. Moreover, one of the pharmacological mechanisms of morphine is inhibition of the adenylate cyclase activity in the cell, thus subsequently lead to suppression of the

activity of protein kinase [8] and finally causes reduced cell excitability [9]. In general, morphine has adverse effect in different organ of the body such as liver, lung, testis, ovary, and brain due to releasing nitric oxide and oxidative effect [10], which that morphine activating lipid peroxidation leads to increasing the reactive oxygen species [11]. In addition, the modern studies illustrated the side effects of this opioid on the reproduction sexual hormones of male and female rats [12]. *Withania somnifera* is a prime medicinal plant [13], [14]. Ultimately, that *W. somnifera* widely used as an immune booster [15], anti-viral [16], natural protective [17], and for multiple medicinal purposes [15], [18].

Synoptically, the *W. somnifera* has antioxidant properties [19], since, the researcher reported that have antioxidant possess and multiple pharmacological activities against toxicity substance, furthermore have anti-inflammatory and hepatoprotective properties [20]. Beside that, a new studies explain that *W. somnifera* has inhibitor effect in the treatment of COVID-19 [21] and improve the immune system by withanolides in COVID-19 infection [15].

Thus, our study aimed for evaluation the productive effect of *W. somnifera* against the adverse effect of morphine on the levels of sexual hormones properties.

Materials and Methods

Experimental animals

The experiment use of 40 adults female Wistar rats, the body weight was ranged between 200 and 250 g. The rats were housed in uniform house conditions and they were given excess amount of food and water in the animal house of the faculty of veterinary medicine in the University of Kufa.

The experimental design

In the present study, the animals divided in the four equal groups as described followed; first group indicated as control group which that received placebo only, while in the second group (indicate as induction group) and third (treatment group) group which both groups received water-soluble morphine for the addictive creation of morphine properties. Moreover, the third group received 6.25% *W. somnifera* extract in new pellets for 21 days. Finally, the fourth group (*Withania S.* group) received 6.25% *W. somnifera* extract in new pellets for 21 day only [22], [23].

For induction rats to morphine addiction in the both induction and treatment groups, the morphine was administered instantaneously at the doses of 0.1, 0.2, and 0.3 mg/mL; each dose was administered for 48 h and finally 4 mg/mL was administered for the remaining 15 days [23].

Preparation of *W. somnifera*

The *W. somnifera* roots separated from the plant and cut into small pieces (1.5 cm) and dried at room temperature for 24 h [24].

Blood sampling collection

Sample was taken immediately from scarified animal heart and then, isolation of the plasma through the centrifuge at 2000 rpm for 15 min and stored in the refrigerator until analysis of the hormonal levels.

Statistical data analysis

Data were analyzed by used SPSS software version 26, used one-way analysis of variance analysis, followed by the *post hoc* Tukey test and statistical test ($p < 0.05$) [25].

Table 1: Protective role of *withania s.* against morphine effects on the level of LH and FSH hormones measurement

Parameters/Groups	Luteinizing Hormone (ng/ml)	Follicle stimulating hormone (ng/ml)
Control group	9.5 ± 0.18 A	14.20 ± 0.15 AC
Morphine group	1.5 ± 0.4 B	20.50 ± 0.5 B
Treatment group	8.9 ± 0.5 A	16.2 ± 0.3 C
<i>Withania S.</i> group	10.1 ± 0.10 A	13.15 ± 0.12 A

Values are means ± SEM of 40 adults male Wistar rats. ABCD, Mean values between groups with different superscripts are significantly different at ($P < 0.05$). Control group; Received placebo only. Morphine group; induction rats to morphine addiction at the doses of 0.1, 0.2, and 0.3 mg/mL each 48 h and finally 4 mg/mL for 15 days, Treatment group; beside the morphine received 6.25% of *Withania somnifera* extract in new pellets for 21 day, *Withania S.* group; received 6.25% *Withania somnifera* extract in new pellets for 21 day only.

Results

The result in the (Table 1) illustrated the adverse effect of the morphine on the hormone regulation of the luteinizing hormone (LH) and follicle stimulating hormone (FSH) as well as showed the productive effect of the *W. somnifera* on the FSH and LH hormones against the addicted effect of morphine in the female rats.

The result of Table 1 showed significant decrease of LH in the morphine groups after 21 days of experimental compared with the control and treatment groups, in addition, exhibited that not significant differences among control and treatment groups that treated by 6.25% *W. somnifera* extract in new pellets for 21 days. Interestingly, that *Withania S.* group exhibited improvement hormonal regulation as showed in both Tables 1 and 2.

Table 2: Protective role of *Withania s.* against morphine effects on the level of progesterone and estrogen hormones measurement

Parameters/Groups	Progesterone (ng/ml)	Estrogen (pg/ml)
Control group	38.2 ± 0.5 A	25.5 ± 1 A
Morphine group	35.2 ± 0.5 A	14.2 ± 0.5 B
Treatment group	36.2 ± 1 A	22.5 ± 0.5A
<i>Withania S.</i> group	39.0 ± 0.3 A	28.0 ± 0.2 A

Values are means ± SEM of 40 adults male Wistar rats. ABCD, Mean values between groups with different superscripts are significantly different at ($p < 0.05$). Control group; Received placebo only. Morphine group; induction rats to morphine addiction at the doses of 0.1, 0.2, and 0.3 mg/mL each 48 h and finally 4 mg/mL for 15 days, Treatment group; beside the morphine received 6.25% of *Withania somnifera* extract in new pellets for 21 day, *Withania S.* group; received 6.25% *Withania somnifera* extract in new pellets for 21 day only.

The result of the Table 2 presented that relapse in the levels of both progesterone and estrogen hormones of morphine group compared with control and treatment groups, although significantly only in level of estrogen as well as treatment group showed significant elevation in the estrogen compared with morphine group.

Discussion

It is a matter of interest that efficacy of morphine in different organs of the body, additionally to that long-term administration of morphine leads to creation addiction habit [4]. The goal of the present study aimed to investigation the protective role of natural source of antioxidant against negative effect of morphine on hormonal regulation.

Morphine has a negative effect on the sex hormonal regulation level due to the harmful effects on the ovary tissue which causes polycystic ovary [26]; researcher illustrated that long-term administration of morphine causes generation free radicals of reactive oxygen and reactive nitrogen [27].

In general, natural plant extracts have antioxidant properties that can improve reproductive system, for example, curcumin [28], thyme [29], and lycopene [30]. The *W. somnifera* considered as antioxidant substance [31] and has different pharmacological activities such as hepatoprotective and anti-inflammatory properties [20].

The findings of the present research suggested that the *W. somnifera* has protective effect against morphine on the regulation of hormone as proved in Tables 1 and 2 which illustrated that promotive and protective effect of *W. somnifera* against noxious effect of the morphine, the result approved and assert by the previous studies, which Karimi *et al.*, 2018 [26] showed that administration of morphine leads to irregularity and disturbance on the hypothalamus pituitary ovary axis and causes anovulatory infertility [32]. Scientists suggested that mechanism effect of morphine is related to the nature of the opioid receptors pharmacokinetic [26]; which morphine has inflammatory effects on the ovary due to causes increase in the secretion of LH in response to GnRH [33], and by inhibit effect of insulin in liver which that stimulate the pancreas for production of insulin [34], then, causes hyperinsulinemia in the polycystic ovary. Finally, it leads to increased levels of androgen production by the ovaries [33].

The results proved the protective effect of *W. somnifera* as illustrated by the previous studies, since proved the regulation effect of the *W. somnifera* due to antioxidant [35] and anti-inflammatory properties which Nasimi *et al.*, 2018 [36] found that *W. somnifera* enhances spermatogenesis in male and sexual behaviors in female; in addition, mechanism of *W. somnifera* perspicuous effect on the reproductive system is antioxidative features and its ability to improve the hormonal balance of LH and FSH and improve detoxification process [37], which plays a main role in inducing gonadotropin releasing hormone secretion [35]. The previous study illustrated the positive effect of *W. somnifera* on histological on different tissue [38], as other extract on the testis tissue testicular and ovary [39].

Conclusion

The present study proved that administration of 6.25% *W. somnifera* extract in new pellets has protective role for enhancement balancing state against the adverse effect of morphine on the levels of sexual hormones properties.

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