Study the Physiological and Histological Effect of Pulmocodin on the Testis of Male Albino Rats

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Abstract

This study was conducted to find out the effect of different doses of Pulmocodin on the physiology of parts of the reproductive system of white male rats. The experiment included administering the Pulmocodin to male white rats by mouth at concentrations (0.325 ، 0.487، 0.650) (Mg/kg), for a period of 3-6 weeks for each concentration. The study recorded the presence of weight changes of the body in addition to physiological changes, as it included results of the experiment were, changes in the levels of hormones (Testosterone, prolactin, LH, FSH) at the middle and end of the experiment.

Keywords*: Pulmocodin, Testis, Albino Rats*

Introduction

the counter drugs are medications that are safe and effective for use by the general public without seeking treatment by a health professional. Popular examples include pain relievers, cough suppressants and antihistamines. Over the counter medicines treat a variety of symptoms due to illness including pain, coughs and colds, diarrhea, heartburn, constipation, acne, and others.OTC drugs have various medical uses and effects, treating mild pain to motion sickness. Some abuse OTC drugs to self-medicate for mental illnesses like anxiety and depression and cough medicines. Abusing these medications can also give users an euphoric "high" or hallucinations. Any use outside of what's recommended is considered abuse. Pulmocodin, one of the OTC drugs made in Iraq, used as cough suppressor and to treat Respiratory tract symptoms, each 5 ml contain: Guaifenesin 100mg, Chlorpheniramine Meleate 1mg, Phenylephrine HCL 5mg and Codeine Phosphate 10mg. Guaifenesin is a glyceryl guaiacolate with expectorant effects [(1)]. Guaifenesin increases respiratory tract mucus secretions, acts as an irritant to gastric vagal receptors and recruits efferent parasympathetic reflexes that cause glandular exocytosis. This agent reduces the viscosity of mucus secretion by reducing adhesiveness and surface tension as well as increasing hydration of mucus. Chlorpheniramine is an antihistamine that reduces the effects of natural chemical histamine in the body and help the body to relax [(2)]. Histamine can produce symptoms of sneezing, itching, watery eyes, and runny nose. Phenylephrine is a medication primarily used as a decongestant, to dilate the pupil, to increase blood pressure, and to relieve hemorrhoids [(3)]. While marketed as a decongestant. Codeine belongs to the class of medications called *narcotic analgesics* It is used to relieve mild-to-moderate pain, anti-cough and diarrhea[(4,5)]. It works by blocking pain signals that are sent out by the brain to various areas of the body. Codeine is also used to control coughing that is not controlled by non-narcotic cough suppressants. It works by acting on the brain to dull the cough reflex. Codeine is one of the opiate drugs that are used to treat acute and chronic pain. While they can be effective when taken as directed, opiates pose a major risk of addiction among their users. The presence of Codeine as one of the main contents led many people abuse Pulmocodin to obtain the euphoria effect and due to it's cheap price of this local manufactured cough drug.

Materials and Methods

Prepare the dose:

The Pulmocodin cough syrup available in the markets and is consumed by adolescents & adults to treat cold symptoms & cough by taking 1 teaspoonful

twice per day , but to get the euphoria effect the dose should be inflated depend on the way of abusing the Pulmocodin by users which is a bottle (100 ml), half of the bottle (50 ml) or a bottle and a half (150 ml). noting that the average weight of the rat was 350 g. The doses were prepared using the following equation:

X / rat weight = dose given / 100

Experiment animals:

56 adult white male rats aged (8-10) weeks, with an average weight of about (360-450 g), were used in this study, obtained from the Animal House of the Cancer Research Center. The animals were placed in plastic cages and their floors were covered with sawdust. The cages were cleaned in terms of replacing sawdust twice during the week, and the cages were sterilized with disinfectants to avoid diseases. During the experiment stages, all animals were subjected to similar laboratory conditions in terms of continuous ventilation and lighting (12 hours of light and 12 hours of darkness), and the temperature ranged between (22-25 ° C) and humidity between (40-60%). Water and suspension were given. Towards continuous duration of the study.

Experimental groups:

The animals were divided randomly into four groups (each group contains 14 animals). the animals were given Pulmocodin with concentrations of (0.325 ، 0.487، 0.650)) mg/kg). Administrated as aqueous solution of the substance orally.

Blood collection:

Blood was collected in two stages (three weeks and six weeks) from the animals by a heart stab method while they were alive. The blood was kept in anticoagulant tubes (EDTA tube), then it was placed in a cooled centrifuge at a rate of 3000 rpm / minute at a temperature of 25◦C for a period of 10 minutes, in order to obtain the serum, after isolating the serum in sterile tubes, tests were performed to see Levels of the following hormones (Testosterone, Prolactin, LH, FSH) were done at the Biotechnology Research Center / Al-Nahrain University.

Statistical Analysis

The Statistical Analysis System- SAS (2012) program was used to detect the effect of difference factors in study parameters. Least significant difference –LSD test (Analysis of Variation-ANOVA) and T-test was used to significant compare between means in this study [6].

Results and Discussion

Changes in Body Weights

Statistical results of the experiment showed a significant increase ($p < 0.05$) in the average body weight after treatment with Pulmocodin (after 3 weeks and 6 weeks) for concentrations (0.325 ، 0.487، 0.650 mg / kg) compared with control group, as shown in table (1). Evidence from both preclinical and clinical studies demonstrates that chronic opioid exposure is associated with increased sugar intake. Preclinical research has attempted to refine the potential pathways and mechanisms of action through which opiates may regulate sugar intake, and how sugar consumption may affect the endogenous opiate system. Preclinical animal studies suggest that direct action of mu agonists at the nucleus accumbens shell, hypothalamus, and paraventricular nucleus is associated with development of sweet preference [7,8]. This process possibly involves GABA-b (G-Protein coupled receptors for gamma-aminobutyric acid)activity in the ventral tegmental area [9]. Consumption of palatable foods, especially on intermittent schedules, is associated with acute binding of the endogenous opiate B-endorphin in the hypothalamus, accumbens shell, cingulated, hippocampus, and locus ceruleus of rats [7,10]. Furthermore, in rats, intermittent access to sucrose leads to decreased enkephalin MRNA production [11]. It is theorized that this down-regulation of enkephalin MRNA production may be associated with increased mu-opiate receptor agonism associated with the rats' sugar intake [12]. The preference for sugary foods resulting from opiate administration may lead to increased consumption of such foods, and possibly accumulation of excess body fat and weight gain.

Table (1): the effect of Pulmocodin for the period (three and six weeks) on the body weights of rats and a comparison of changes between the first and second periods.

Group	Mean ± SE of Body weight (g)		
	Before dosage	Period 1	Period 2
Control	368.50 ± 3.50 b	389.00 ± 2.00 d	421.00 ± 4.00 c
LD: 0.325 ml	380.00 ± 2.00 b	420.50 ± 1.50 c	485.00 ± 3.00 b
MD: 0.487 ml	387.50 ± 7.50 b	438.50 ± 1.50 b	502.50 ± 12.50 b
HD: 0.650 ml	410.00 ± 5.00 a	512.00 ± 2.00 a	591.00 ± 11.00 a
LSD value	19.385 *	6.941 **	34.132 **
P-value	0.0267	0.0001	0.0008
Means having with the different letters in same column differed significantly. * (P≤0.05), ** (P≤0.01).			

The means that carries different letters within the same column differs significantly between them. n represents the number of animals. * (P≤0.05), NS: Non-Significant

The effect on the level of hormones in male rats:

Testosterone:

In this experiment, a significant increase (P≤0.01) was found in the testosterone concentration of animals treated with Pulmocodin and for concentrations (0.325 ، 0.487، 0.650 mg / kg) after three weeks, and it did not show a significant difference after six weeks, as shown in the table (2). As Pulmocodin may be couldn't have an effect on testosterone through there is no effect on Leydig cells. In the rat, the fetal Leydig cells begin to produce testosterone by gestational day 15.5, with peak production just prior to birth. In the mouse, the fetal Leydig cells produce androstenedione which is then converted into testosterone by the fetal Sertoli cells [13,14,15].

Table (2): The effect Pulmocodin for period (three and six weeks) on the Testosterone concentration in rats, and a comparison of changes between the first and second period.

Group	Mean ± SE of Testosterone (ng/dI)	
	Period 1	Period 2
Control	2.30 ± 0.10 d	2.30 ± 0.10 b
LD: 0.325 ml	2.77 ± 0.02 c	2.31 ± 0.05 b
MD: 0.487 ml	5.04 ± 0.02 b	1.25 ± 0.25 c
HD: 0.650 ml	7.68 ± 0.02 a	3.78 ± 0.03 a
LSD value	0.2045 **	0.5418 **
P-value	0.0001	0.0001
Means having with the different letters in same column differed significantly. ** (P≤0.01).		

The means that carries different letters within the same column differs significantly between them. n represents the number of animals. * (P≤0.05), NS: Non-Significant

Prolactin hormone:

In the first three week, the results showed a significant decrease (P≤0.01). in the concentration of prolactin hormone for animals treated with Pulmocodin for concentrations (0.325 ، 0.487، 0.650 mg / kg) during the first period compared to the control group, but after six weeks an increase of the concentrations of Prolactine hormone for animals treated with Pulmocodin, this may be related to the relationship between the two hormones testosterone and prolactin is close as shown in table (3).

Table (3): The effect of Pulmocodin for period (three and six weeks) on the prolactin concentration in rats, and a comparison of changes between the first and second Period.

Group	Mean ± SE of PRL (ng/ml)	
	Period 1	Period 2
Control	2.100 ± 0.100 a	2.100 ± 0.100 a
LD: 0.325 ml	1.015 ± 0.005 b	1.005 ± 0.005 b
MD: 0.487 ml	1.015 ± 0.015 b	1.010 ± 0.010 b
HD: 0.650 ml	1.200 ± 0.200 b	2.140 ± 0.140 a
LSD value	0.4401 **	0.3385 **
P-value	0.0063	0.0001
Means having with the different letters in same column differed significantly. ** (P≤0.01).		

The means that carries different letters within the same column differs significantly between them. n represents the number of animals.

*** (P≤0.05), NS: Non-Significant.**

Luteinizing hormone (LH):

In this experiment, a significant increase (P≤0.01) was found in the LH hormone concentration of animals treated with Pulmocodin and for the concentrations (0.325 ، 0.487، 0.650 mg / kg) during the six-week period as shown in the table)4). Although, the previous study observed that chronic codeine administration in rabbits led to enhanced sexual motivation and copulatory locomotor activity, as well as reduced copulatory efficiency, fertility index, and serum level of testosterone

Table (4): The effect of Pulmocodin for period (three and six weeks) on the LH concentration in rats, and a comparison of changes between the two periods.

Group	Mean ± SE of LH (IU/L)	
	Period 1	Period 2
Control	2.00 ± 0.10 b	2.00 ± 0.10 b
LD: 0.325 ml	4.12 ± 0.02 a	1.88 ± 0.11 b
MD: 0.487 ml	1.005 ± 0.01c	1.00 ± 0.00 c
HD: 0.650 ml	1.125 ± 0.03 c	5.46 ± 0.06 a

Cont... **Table (4): The effect of Pulmocodin for period (three and six weeks) on the LH concentration in rats, and a comparison of changes between the two periods.**

LSD value	0.2064 **	0.3181 **
P-value	0.0001	0.0001
Means having with the different letters in same column differed significantly. ** (P≤0.01).		

The means that carries different letters within the same column differs significantly between them. n represents the number of animals.

* (P≤0.05), NS: Non-Significant

Follicle stimulating hormone (FSH):

In the first three weeks, the results showed non-significant differences in the FSH concentration of animals treated with Pulmocodin and for concentrations (0.325 ، 0.487، 0.650 mg / kg) compared to the control group.

In the six weeks period, a significant increase (P≤0.05), was found in the FSH concentration of animals treated with Pulmocodin and for concentrations (0.325 ، 0.487، 0.650 mg / kg) as shown in the table (5). The increase of FSH hormone concentration is may be due to the hypothalamic–pituitary axis is constantly under the effect of multiple substances including neurotransmitters, steroid hormones and endogenous opioids. Exogenous opioids exert an effect on the same receptors as endogenous opioids and have been shown to interfere with the release (including its pulsatile nature) of GnRH [16,17] It has been suggested that morphine inhibits the biosynthesis of GnRH.(17) Opioids also decrease the negative feedback of sex steroids on the anterior pituitary, as well as its response to GnRH. [16] In contrast, FSH secretion is not, or only minimally, affected.

Table (5): The effect of Pulmocodin for period (three and six weeks) on the FSH concentration in rats, and a comparison of changes between the first and second Period.

Group	**Mean ± SE of FSH (IU/L)**	
	Period 1	**Period 2**
Control	1.010 ± 0.010 a	1.010 ± 0.010 b
LD: 0.325 ml	1.005 ± 0.005 a	1.015 ± 0.015 b
MD: 0.487 ml	1.010 ± 0.010 a	1.015 ± 0.015 b
HD: 0.650 ml	1.005 ± 0.005 a	1.070 ± 0.010 a
LSD value	0.031 NS	0.0501 *
P-value	0.9352	0.0476
Means having with the different letters in same column differed significantly. * (P≤0.05), NS: Non-Significant.		

The means that carries different letters within the same column differs significantly between them. n represents the number of animals.

* (P≤0.05), NS: Non-Signific

Conclusion

High doses of Pulmocodin are considered misuse of the drug because it is used more than the recommended dose, as Pulmocodin affected body and Testis as well as epididymis weight by increasing concentrations and the dosing period, also Pulmocodin affected fertility of males rats, as the number of births decreased with the increase in concentration and dosing period.

Conflict of Interest: None

Funding: Self

Ethical Clearance: Not required

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