



# Effect of different concentrations of Nandrolone decanoate on weights the testes and Epididymis of male white mice infected with the protoscolices of the *Echinococcus granulosus*

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## Abstract

This study was conducted in the Animal House of the Department of / Biology / Faculty of Education for girls to detect some of the side effects associated with the use of Nandrolone in the weights of testes and Epididymis of infected with mice protoscolices through the use of (50) male Swiss mice Balb / c age Three months were randomly divided into five equal groups: positive control group, negative control group, and the remaining three groups were injected with concentrations (0.1, 0.2 and 0.3 mg / g) of Nandrolone. The study showed a significant decrease ( $P < 0.05$ ) in the weights of testes and Epididymis of mice Treatment compared to the positive control group and the negative group. The study also showed that the third concentration (0.3) mg / g of Nandrolone has shown several side effects on certain organs related to the male reproductive system.

**Keywords:** HCD: Hydatids cystic disease, Nandrolone decanoate

## INTRODUCTION:

Hydatids cystic disease, which is widespread in the world, including the Arab world and Iraq, and the incidence of this disease by ingesting the eggs of the *Echinococcus granulosus* with foods that are contaminated with the stool of the final host (Felidae) after hatching eggs onchospher grow and penetration the intestinal wall. The liver is often the first organ for infection<sup>[1]</sup>. It is one of the most danger epidemiological health problems in most of the world and is a common disease between humans and animals. The disease in humans and other intermediates (sheep, cows, buffaloes, camels, horses and other animals) results from the larval stage of Cestoda parasitic belonging to the genus of *Echinococcus*, which includes many species, most notably medical genus *E.granulosus* and vesicular species *E.mutiocularis*. This disease in Iraq is still endemic and socially and economically affected, as well as its effects on the human health aspect, which led many researchers to investigate the treatment methods.( Reference) Hence the importance of using some drugs and some plant extracts that may help in treating the disease. The infestation of parasitic *Echinococcus granulosus* can affect fertility as a result of parasitic changes in the testicular and Epididymis tissue, vector vessels and prostate in infected males, in addition to its effect on The main reproductive parameters are sperm movement, concentration, and vitality, which have the greatest role in the reproductive capacity of males.(Reference) Nandrolone decanoate is an anabolic steroids and is a derivative of testosterone, which belongs to Estran derivatives but is no different in that it does not have the methyl group in the carbon atom (10), As well as group17 hydroxyl  $\beta$ , Nandrolone decanoate from steroid compound with long-term physiological effects, and can be made chemically, but it exists naturally in some types of mammalian compared to hormone testicular fat testosterone, Nandrolone decanoate has anabolic capacity five times greater than those shown by testosterone, but the effectiveness is less androgenic and higher Toxicity, And this has been demonstrated by the results obtained from some animal biopsies, and many studies have indicated that the use of high doses of Nandrolone may cause carcinogenic and other hereditary effects in animals<sup>[2][3]</sup>, And for the therapeutic properties of Nandrolone decanoate it is used in the treatment of osteoporosis in postmenopausal women<sup>[4]</sup> and works to increase the weight and mass of the body in patients with HIV<sup>[5]</sup>. In the treatment of prostatic hyperplasia and cancer<sup>[6]</sup>, Excessive use of Nandrolone decanoate directly disrupts the efficacy of endocrine glands or its cumulative effects as well as other fertility problems due to its high ability to alter the function of male genital organs<sup>[7]</sup>.

## MATERIALS AND METHODS

### Preparation of the laboratory animals

This study included of 50 adult male of mice - three months old - of Balb / c type *Mus musculus*, and were placed in the Animal House of the Department of Biology / Faculty of Education for Girls, which had all laboratory conditions of light (13) (11) hours of darkness) and temperature (23 – 28C°) and animal feed for special nutrition, and divided to five groups of males (10) males per group, three groups of which were used to study the effect of Nandrolone, The fourth group as the negative control group and the fifth group. The positive control group, after knowing the appropriate number of live protoscolices in a certain volume of the protoscolices suspension and Phosphate Buffered Solution, injected 2,000 protoscolices (0.7 ml) with a 1 ml syringe and measuring needle 21 degrees in the Intraperitoneal(I.P.) after sterilization the injection area by alcohol 70% at injection of each mice of the experimental mice<sup>[8]</sup>.

### Solutions and Stains used

#### Normal Saline solution

This solution was prepared according to the method of <sup>[9]</sup>, which was used to wash the wall of the Hydatid Cyst to collect suspended Protoscolices.

#### Phosphate Buffered Solution (PBS)

This solution was prepared according to<sup>[10]</sup>, which was used to wash the generating layer of Hydatid cyst.

#### Kreb's – Ringer's Solution (K.R.S.)

This solution was prepared by<sup>[11]</sup>. Then, the sterilized Kreb's - Ringer's Solution was mixed with Hydatid cystic fluid (HCF) in ratio of 4:1. This solution was used to measure the vitality of Protoscolices and to preserve them. This solution is considered as one of the best mediums for keeping the Protoscolices alive outside the living body after being isolated from the Hydatids Cysts<sup>[12]</sup>.

#### Aqueous Eosin Stain

This stain was prepared by dissolving (0.1) g of stain powder in (100) ml of distilled water according to the method of <sup>[13]</sup>. This stain was used to measure the vitality of the Protoscolices.

#### Collection of Hydatids Cysts and Preparation of Protoscolices.

The samples of Hydatids Cysts were collected from naturally infected sheep livers in abattoir of Al-Najaf Al-Ashraf Governorate, as the samples in special containers made of polystyrene, chilled with crushed ice, to preserve the vitality of the Protoscolices. They were treated directly and transferred to the advanced parasite laboratory at the Faculty of Education for Girls, University of Kufa, and as soon as the infected organs were brought directly to the laboratory, the containers were opened

directly to confirm the existence of the Protoscolices inside them. The vitality of the Protoscolices was examined by using (Microscope or ...) the Aqueous Eosin Stain with concentration of (0.1%). The organs were washed with water for the purpose of removing blood and suspended materials during slaughter. The outer surface of the Hydatid cysts was then sterilized with a piece of cotton moistened with Ethyl Alcohol (70%), and the cyst fluid was then withdrawn by a plastic syringe with a size of (10) ml with a needle measuring (21) degrees. The collection process was performed under sterilized conditions, The Hydatids cysts fluid was withdrawn with the Protoscolices and placed in a 500 ml glass beaker. The cysts were then opened by using surgical scissors and forceps. The infecting parasitic layer was extracted and placed in a sterilized container consist of physiological sterilized saline solution, and then washed by a washing bottle containing sterilized Phosphate Buffered Solution (PBS) with pH equal (7.2) for several times to extract many Protoscolices as possible.

This suspension was collected after the washing process and was added to the pre-drawn cystic fluid and the Protoscolices were placed in sterilized test tubes for Sedimentation with Centrifuge, three times at 3000 cycles/minute for 15 minutes, Before the start of the second wash (Procaine Penicillin by 2000 IU/L and Streptomycin by one g/L were added to the washing solution In the second wash PBS, and after completion of the Sedimentation process by centrifuge. The filtrate was poured and a small amount of sterilized Phosphate Buffered Solution was added to the precipitate and the calculation of the Protoscolices was performed. The fluid was collected in other sterilized bottles covered with Paraffin wax and stored in the refrigerator to use later. The suspension of Protoscolices was preserved with a Kreb's – Ringer's Solution + Hydatid cystic fluid HCF(1:4), followed by Viability test and calculate the number of Protoscolices<sup>[14]</sup>.

**Estimation of the Protoscolices Viability**

Method was depended<sup>[15]</sup>. The number of five repeats was depended in the determination of the viability proportion, which reached 94.7%.

**Preparation of concentrations of Nandrolone**

Prepare three sequential Nandrolone concentrations for the current research (0.1, 0.2 and 0.3 mg / g, respectively). It was obtained from company China, Meheco corporation, Beijing, China.

**Anatomy of animal**

The animal weights of this study were recorded by using a medical weight scale. The animals were anesthetized using diethyl ether. Then removed testes and Epididymis and removed adherent fatty substances and dried by filter paper. Then recorded weights by a sensitive electronic scale.

**Statistical analysis**

The results of this study were analyzed statistically, using t ANOVA test. Thus, the differences between the rates of the current survey were extracted by using the least significant difference (L.S.D) at the statistical level (  $P < 0.05$  )<sup>[16]</sup>.

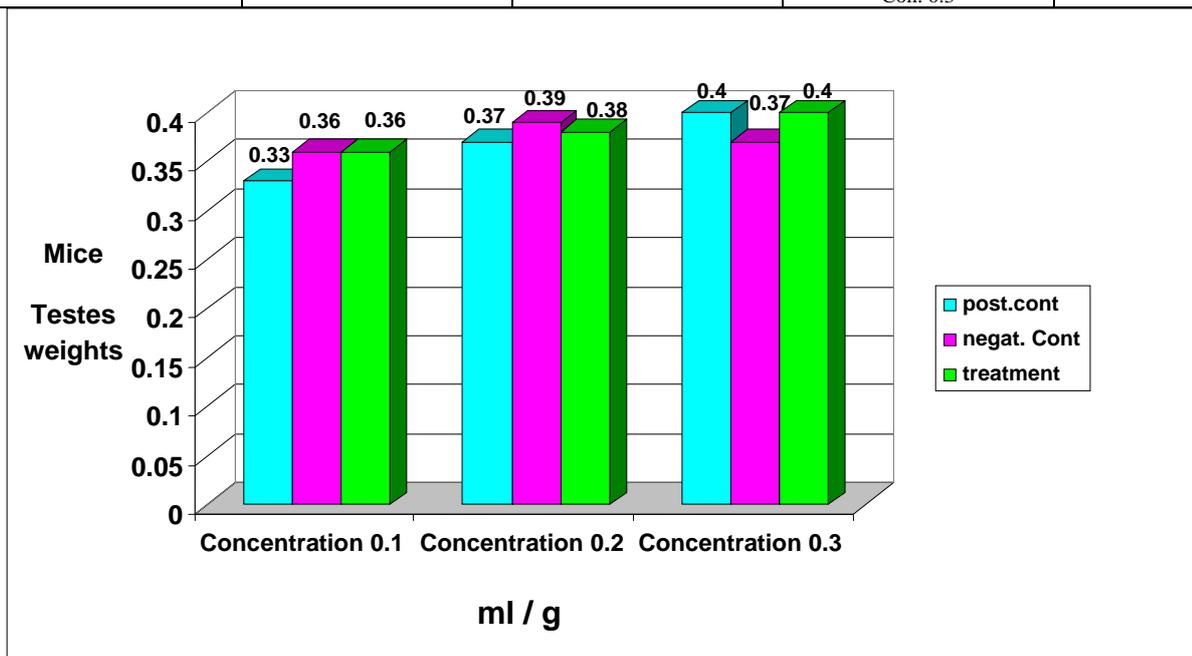
**RESULTS:**

**Effect of Nandrolone decanoate In weights of the testes and Epididymis**

Table 1 showed a significant decrease ( $P < 0.05$ ) in weight of the testes and Epididymis by the injection effect of the third concentration of Nandrolone compared to the control group, but the first and second concentrations did not show any significant difference when measured in that group. Significant differences were observed ( $P < 0.05$ ) when comparing the third concentration with the first and second concentrations of the Nandrolone.

**Table (1): Effect of Nandrolone in Mean of testes weights of laboratory**

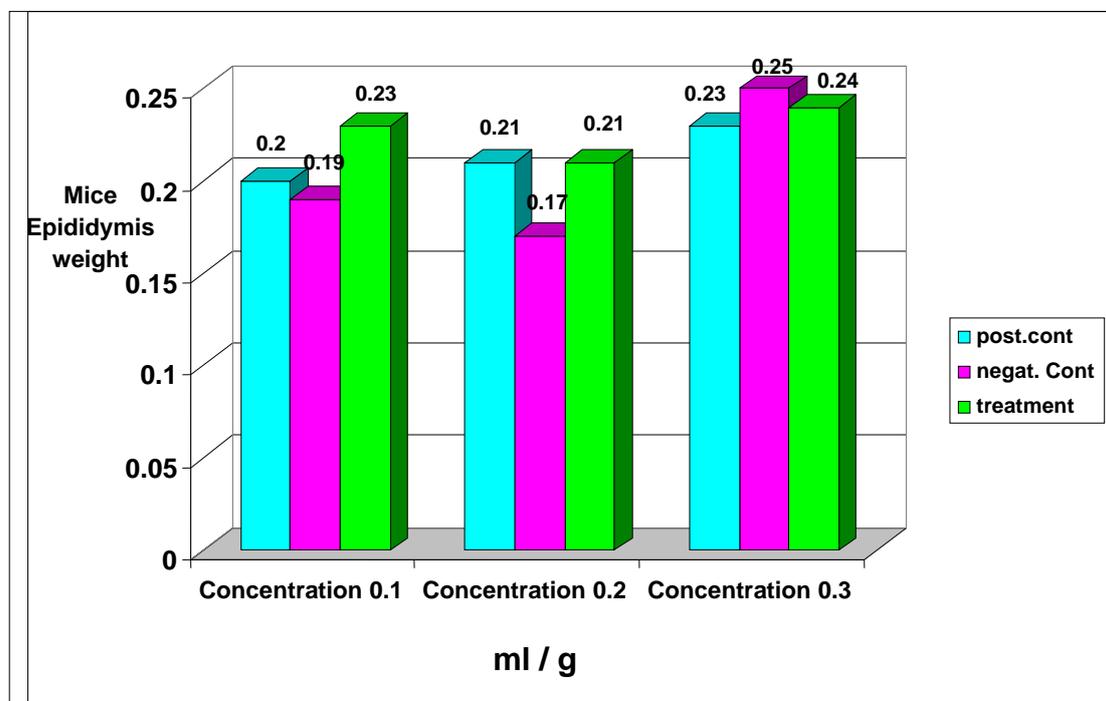
Drugs Concentrations mg/g	Positive control M ± SE	Negative control M ± SE	Treatments M ± SE	T test $P < 0.05$
0.1	0.33 ± 0.01	0.36 ± 0.01	0.36 ± 0.02	1.5 non sign
0.2	0.37 ± 0.01	0.39 ± 0.01	0.38 ± 0.01	0.63 non sign
0.3	0.4 ± 0.01	0.37 ± 0.01	0.4 ± 0.01	non sign
L.S.D			0.03 Sign Con. 0.3	



**Figure (1) Effect of Nandrolone in the Mean of testes weights of laboratory mice**

**Table (2) Effect of Nandrolone in the Mean of Epididymis weights of laboratory mice**

Drugs Concentrations mg/g	Positive control M ± SE	Negative control M ± SE	Treatments M ± SE	T test P < 0.05
0.1	0.2 ± 0.01	0.19 ± 0.01	0.23 ± 0.01	2.4 sign
0.2	0.21 ± 0.01	0.17 ± 0.01	0.21 ± 0.01	0 non sign
0.3	0.23 ± 0.02	0.25 ± 0.01	0.24 ± 0.02	0.5 non sign
L.S.D			non Sign	

**Figure (2) Effect of Nandrolone in the Mean of Epididymis weights of laboratory mice**

#### DISCUSSION

Decrease in body weight was consistent with some studies<sup>[17]</sup>, but differed with other studies<sup>[18] [19]</sup>, who reported that the treatment of male mice with Nandrolone did not occur a significant change in body weight.

Decrease in weight may be attributed to the possibility that injection of male mice with a concentration of 0.3 mg / g of the hormone may cause disturbances in the gastrointestinal tract resulting in a decrease in the mice of consumption of the diet due to its bad digestion or loss of appetite for food. High doses of Nandrolone increase the risk of upper gastrointestinal disorders and gastric hemorrhage<sup>[20]</sup> And this agree with current study, which negatively affected their weight and decreased significantly. The decrease in weight is likely to be due to a decrease in the concentration of testosterone due to the effect of androgens, especially the testosterone which is the president of male hormones in increasing body weight because of its structural action on skeletal muscle, stimulating protein synthesis and increasing the weight and size of the kidneys, Or perhaps the decline in weight in part to the moral decline in the weights of testes and Epididymis of animals treated with the hormone Nandrolone, which was recorded during the current study, as they form an integral part of the overall structure of the body so it is possible that the decline in weight has negatively affected weight General for male mice.

The observed reduction in the weights of the testis and the Epididymis was consistent with some studies<sup>[21]</sup>, which may indicate a decrease in the diameter of the Seminiferous tubules in the testis, as well as in the epidermal regions of the epididymal tubules and the rise of epithelial cells in the head of the Epididymis. In the reduction of weights of testes and Epididymis

for treated mice, as these tubules form the main core (structural and functional unit), for both the testis and the Epididymis. It is possible that the decrease in the weights of the testes and the Epididymis resulted from pathological changes in the histological structure of each of them was induced by the injection of concentration (0.3) mg / g of Nandrolone hormone, which may have had an effective effect in the reduction of their weight significantly in treated males, The use of high doses of Nandrolone causes impaired efficiency or reproductive capacity<sup>[22]</sup>, and that the drug's pathogen originates from an oxidative oxidation product (NAPQ)<sup>[23]</sup>.The reduction in testes weights in male hormone-treated mice may indicate the inhibitory activity of the drug to inhibit the secretion of FSH and ICSH from the frontal lobe of the pituitary gland, which may cause testicular atrophy and significant weight loss. Some studies have indicated that injecting mice with high concentrations Of the hormone Nandrolone occurs in the uterus and ovaries in the female and in the testes in males<sup>[24]</sup>, and a subsequent study that the use of high concentrations of the hormone may impair or adversely affect the performance of natural reproduction for the action against the nutrients of the vaccine and inhibits manufacturing Vital to Prostaglandin<sup>[25]</sup>.

The reduction in the weight of the Epididymis (head and tail) in treated males is likely to be due to the effect of concentration (0.3) mg / g of the hormone at the level of testosterone and its reduction, which would lead to a significant reduction in the weights of these standards, That testosterone fat contributes effectively to the development of seminal vesicles, ducts and epididymis in human male embryos, while the development of the testis and scrotum and penis under the influence of Dihydrotestosterone<sup>[26]</sup>. It is also that the hormone testosterone is

necessary and essential for the construction of tissue and functional performance of the subsequent sex glands.

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