ISSN No:-2456-2165

Evaluation of Male Reproductive Hormones in Reserpine Treated Rabbits

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Abstract:- **Depressive disorders are extremely common mental disorders with a life-time prevalence of about 16%. Estimations have shown that by the year 2020 depressive disorders would be the second most significant contributor to the impairment of health globally. This paper is aimed at evaluating the role of reserpine on male reproductive hormones in rabbits. Ten sexually matured rabbits were used for the study, animals were divided into two groups (n=5). Group A (control) was given distilled water, Group B was given reserpine. Blood was collected for via cardiac puncture of the heart and various organs like epididymis, corpus carvenosa and testes were harvested for histological analysis. Data obtained were analyzed using Student's T-test and $p<0.05$ was considered significant. The result obtained showed significant reduction in serum testosterone level, while serum LH and FSH level remained unchanged. Epididymal weight result showed significant decrease while testicular weight and corpus carvenosa remained unchanged. The cytoarchitecture of testicular tissue revealed severe damaged to the leydig cell, while that of penile tissue showed loosened connective tissues in corpus spongiosum. This study therefore confirms that reserpine alters serum testosterone levels in male rabbits but the mechanism still remains unclear.**

Keywords:- *Depression; Erectile Dysfunction; Testosterone; Luteinizing Hormone; Follicle Stimulating Hormone; Epididymis.*

I. INTRODUCTION

Depression is a leading cause of morbidity worldwide [1]. Depression is highly prevalent [2] and has a profound impact on functioning and quality of life [3]. It is a serious mood disorders and affects up to 20% of the global population [4]. Those suffering from depression are at higher risk of diseases usually associated with increasing age such as cardiovascular disease [5], obesity [6], diabetes, cancer [7], and cognitive impairment [8] and have a higher all-cause mortality rate [9].

Depressive disorders are extremely common mental disorders with a life-time prevalence of about 16%. Estimations have shown that by the year 2020 depressive disorders will be the second most significant contributor to the impairment of health globally [10]. Till date, no specific patho-physiological process has been linked to the neuronal morphological changes that go along with this disease. One of the most plausible causes for these neuronal alterations is elevated oxidative stress due to increased production of free radicals. Various evidences within the last decade, both in humans and also preclinical findings from animal models support this "oxidative stress hypothesis of depressive disorder" [11] [12].

Normal sexual function is a biopsychosocial process. Sexual dysfunction may be biologic, psychologic, or social in origin, but mostly affects the three.

Depression and erectile dysfunction (ED) clearly are associated [13]. In a landmark clinical study conducted in Massachusetts, men who had untreated depression were almost twice as likely to report ED as men who did not have depression [14]. Various symptoms of depression include feelings of sadness and hopelessness, loss of interest in pleasurable activities (anhedonia), changes in appetite, disturbance of sleep, fatigue, and inability to concentrate [15]. Numerous clinical studies have documented an association between depression and ED because loss of interest in pleasurable activities, including sex, is a diagnostic criterion of depression. It is not surprising that sexual dysfunction is common in men who have depression; low libido is most prevalent, followed by orgasmic difficulty and finally ED, which generally is considered an arousal disorder [16]. Erectile dysfunction (ED) is a common disorder of aging men with a prevalence of 5% in men 40 years of age, increasing to 15% to 25% at age 65 years and older [17]. Interestingly, major depressive disorder is frequently associated with decreased libido, diminished erectile function, and decreased sexual activity [18] [19]. In some men, the presence of depressive disorder is associated with a reversible impairment in sexual neurophysiology, leading to ED. Hypogonadal men exhibit a significantly higher prevalence of anxiety disorders and major depressive disorder, compared to those with normal physiological levels of androgens [20] [21]. Similarly to hypogonadal men, rodents with low testosterone levels can exhibit increased depressive-like behaviors.

Reserpine is an antihypertensive drug origin from the roots of certain species of Rauwolfia, usually R. serpentina or R. vomitoria, and it can also be synthesized. It is white or pale bluff in colour, insoluble in water, freely soluble in chloroform and acetic acid. Reserpine inhibits the uptake of norepinephrine into storage vesicles resulting in depletion of cathecolamines and serotonin from central and peripheral axon terminals. Its chemical name is methyl 18β-hydroxy-11, 17αdimethoxy-3β, 20α-yohimban-16β-carboxylate-3,4,5-trimethoxybenzoate (ester).

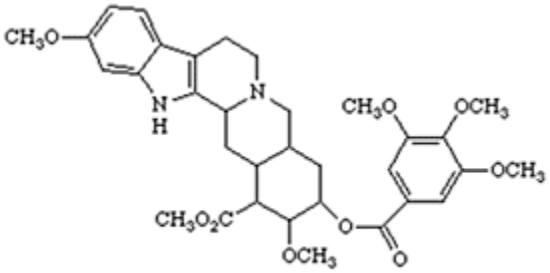

Fig. 1. Structural Formula of Reserpine

The main risks associated with reserpine toxicity are central nervous system depression, the development of psychiatric depression, cardiovascular toxicity and gastrointestinal irritation. Reserpine inhibits normal sympathetic activity in both the CNS and peripheral nervous system by binding to cathecolamines and serotonin in nerve cells which result to cathecolamine depletion. The physiological mechanism involved in normal sexual response includes neurogenic, psychogenic, vascular and hormonal factors that are coordinated by centers in the hypothalamus, limbic system and cerebral cortex. Sexual dysfunction is frequently attributed to antihypertensive and antipsychotic agents and is a cause of non-compliance. Drug induced effects include diminished libido, ejaculatory disturbances, impotence, delayed orgasm etc. The most frequent is impotence. These effects may be due to adrenergic inhibition, adrenergic-receptor blockage, endocrine and sedatives effects and anticholinergic properties of these drugs.

Although several studies have revealed the relationship between various antidepressants drugs and reproductive functions, but little is known on how depression causes erectile dysfunction. Hence, this study aimed to evaluate the effect of reserpine treatment on male reproductive hormones in rabbits.

This research is aimed at evaluating male reproductive hormones in reserpine treated rabbits. It focuses on determining the effect of reserpine on body weight and organ weight of male rabbits; the effect of reserpine on lutenizing hormone (LH) and follicle stimulating hormone (FSH); the effect of reserpine on serum testosterone level; and the cyto-architecture of testes and penile tissue in male rabbits.

II. EXPERIMENTAL SECTION

A. Animals

Ten healthy sexually (10) mature male rabbits (1.5-2.5kg) were used for this study. Animals were purchased from Onileola farms in Ede, Osun state. All animals were housed at the central animal house in the Faculty of Basic Medical Sciences, Osun State University Osogbo in a conducive environment. These animals were kept in well ventilated cages which are kept clean on regular basis. Throughout the experiment, the animals were maintained in a 12hours/12hours dark cycle under relatively constant temperature and were fed with standard feed and given free access to water *ad libitum.*

B. Drug Preparation and Administration

Reserpine which was purchased in TNJ Chemicals, China was used for the study. The dosage was freshly prepared in a calculated volume of distilled water to form stock solution and administered at1 mg/kg. The preparation was done in the Physiology Laboratory of the College of Health Sciences, Osun State University. Body weight of animals was monitored and recorded throughout the experiment. The appropriate dosage of reserpine corresponding to the weight of an individual rabbit is drawn and administered via oral galvaging with oral canula. Administration was carried out for 3 days.

C. Experimental Design

Rabbits were accommodated to the laboratory conditions two weeks for acclimatization prior to the commencement of the study. These rabbits were randomly divided into two groups of five animals each (n=5). Animals were divided into experimental and control groups. The experimental groups were administered reserpine, while the control groups were given distilled water daily.
Group A: 5 rabbits that received 1ml of distilled water.
Group B: 5 rabbits that received reserpine (1 mg/kg).

D. Organ Collection

The animals were dissected and the organ of interests; testes, corpus carvenosus and epididymis, were removed, cleared of adherent tissues and weighed immediately using an electronic weighing balance.

E. Histological Analysis

Testes and corpus carvenosa from each of the groups were taken immediately the animals were opened up. The selected tissue was sliced, placed in a tissue container and properly labeled. They were fixed in Bouin's fluid before they were transferred into 10% formal saline so as to pressure the various constituents in their normal micro-anatomical positioning and prevent them from any degeneration or analytic changes.

F. Immunoassay

Serum was obtained from spun blood by separating from the cells using Pasteur pipette into another plain serum bottle and stored in ice packs. Then the hormonal assay for serum concentration of testosterone, follicle stimulating hormone, luteinizing hormone and nitric oxide was carried out.

G. Statistical Analysis

Data obtained were expressed as Mean ±SEM and analyzed using Student's T-test, $p<0.05$ was considered significant.

III. RESULTS AND DISCUSSION

A. Effects of Reserpine on Mean Body Weight of Male Rabbits

The result obtained from this study showed no significant difference in the initial and final body weight of control as compared with the reserpine treated group. The control group shows a slight increase in initial body weight

ISSN No:-2456-2165

and a slight decrease in final body weight, while reserpine treated group showed a slight increase in the initial body weight and a slight decrease in final body weight.

Table I: Mean Body Weight of Male Rabbits

TREATMENT	INITIAL WEIGHT (KG)	FINAL WEIGHT (KG)
CONTROL	1.720 ± 0.1020	1.910 ± 0.03317
RESERPINE	1.880 ± 0.1497	1.710 ± 0.08124

Data presented as Mean ±SEM,
*P<0.05 was considered significant when compared with control

B. Effects of Reserpine on Weight of Reproductive Organs of Male Rabbits

The result showed no significant difference in the testicular weight of both groups when compared, though there is a slight increase in the testicular weight of reserpine treated group. The corpus carvenosa weight showed no significant difference in both groups as compared while the epididymal weight of the control group showed significant increase when compared with the reserpine treated group.

Table II: Weight of Reproductive Organs of Male Rabbits

TREATMENT	TESTES (g)	CORPUS CARVE NOSUM (g)	EPIDIDYMAL (g)
CONTROL	0.08399 ± 0.01980	0.03286 ± 0.002688	0.1267 ± 0.03388
RESERPINE	0.1220 ± 0.01073	0.03195 ± 0.001916	0.04724 ± 0.005069*

Data presented as Mean ±SEM,
*P<0.05 was considered significant when compared with control

C. Effects of Reserpine on serum testosterone levels in male rabbits.

The result obtained in this study showed significant reduction (p<0.05) in serum testosterone levels of reserpine treated group as compared to that of the control.

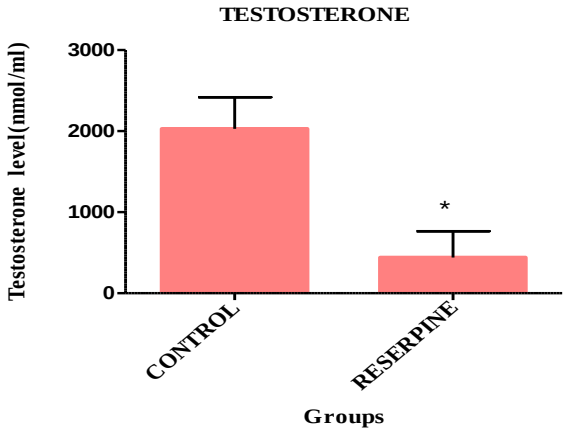

Fig 2: Serum Testosterone Levels in Male Rabbits.
Data presented as Mean ±SEM
*P<0.05 was considered significant when compared with control

D. Effects of Reserpine on Serum Luitenizing Hormone Levels (LH) in Male Rabbits

There was no significant difference in serum luteinizing hormone levels in reserpine treated group when compared with the control but there is a slight reduction in the serum LH levels of reserpine treated group.

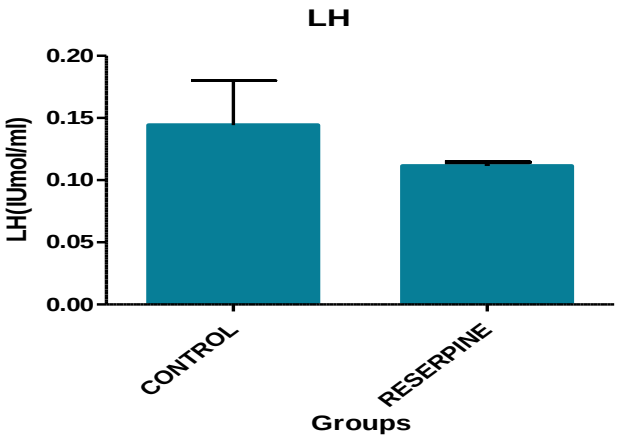

Fig 3: Serum Luitenizing Hormone levels (LH) in Male Rabbits
Data presented as Mean ±SEM
*P<0.05 was considered significant when compared with control

E. Effects of Reserpine on Serum Follicle Stimulating Hormone (FSH) in Male Rabbits.

This study showed no significant difference in FSH levels of reserpine treated group (p<0.05) as compared with control.

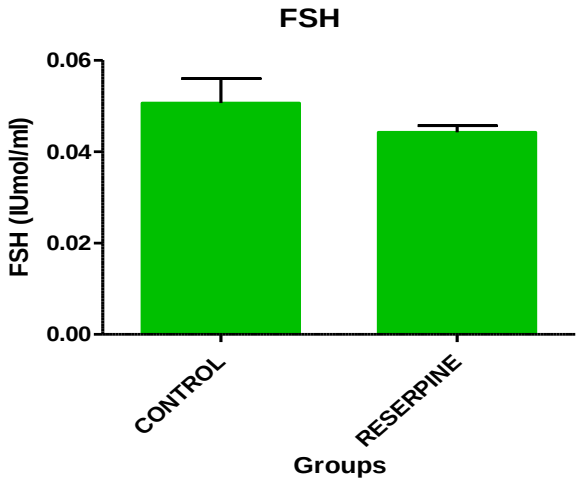

Fig 4: Serum Follicle Stimulating Hormone (FSH) Levels in Male Rabbits
Data presented as Mean ±SEM
*P<0.05 was considered significant when compared with control

F. Effects of Reserpine on Histology of the Testes and Penile Tissues in Male Rabbits

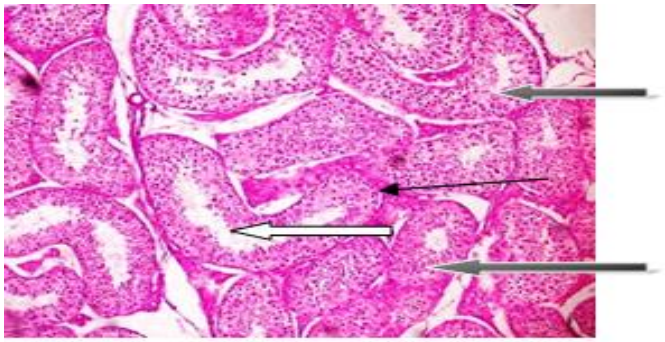

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Fig 5: Photomicrograph of control (Testis)

In Fig 5, the photomicrograph of control group testicular section stained by haematoxylin and eosin shows it is normal. The seminiferous tubules are intact and leydig cells appear normal.

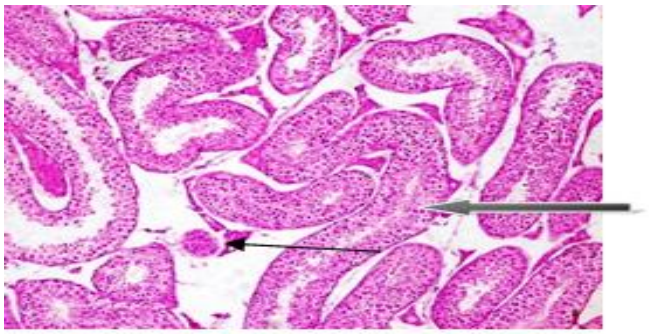

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Fig 6: Photomicrograph of Reserpine Treated Testis

In Fig 6, the photomicrograph of a testicular section stained by haematoxylin and eosin shows maturation arrest; hypospermatogenesis (black arrow) - there are several seminiferous tubules showing reduction in germ cell elements development and incomplete developmental stages, there are short luminal diameters lacking spermatocytes. The interstitial spaces show leydig cells hyperplasia (slender arrow).

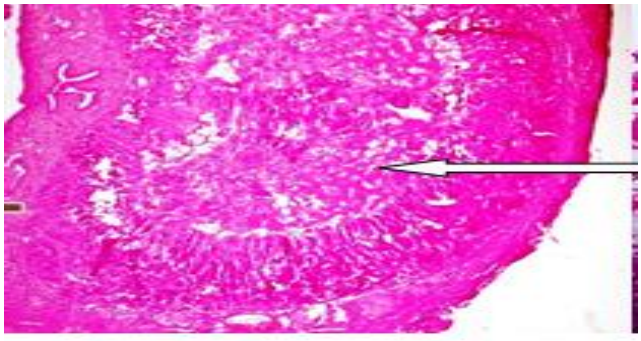

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Fig 7: Photomicrograph of control (Penile Tissue)

In Fig 7, the photomicrograph of control group penile tissue stained by Haematoxylin and eosin shows normal histomorphology, the glans shows normal connective tissues in corpus spongiosium(white arrow). The corpus carvenosa seen contain interstitial fibrous connective tissue.

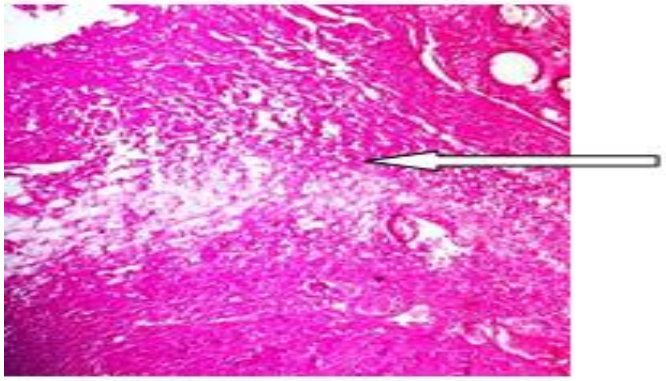

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Fig 8: Photomicrograph of Reserpine Treated Penile Tissue

In Fig 8, the photomicrograph of a penile tissue stained by Haematoxylin and Eosin showing poor histomorphology; the glans shows loosened connective tissues in corpus spongiosum (white arrow), and there is moderate lipid deposition and focal area of mild edema. The Corpus cavernosum seen contains very loose interstitial fibrous connective tissue.

Results obtained from this study revealed that reserpine has a reducing effect on body weight, which has also been reported by [22]. The observed decrease in body weight might be due to loss of appetite exhibited by the reserpine treated rabbits following depressive-like symptoms.

The organ weight was also reduced in reserpine treated rabbits, though that of testes and corpus carvenosa decrease slightly but not significant while there was significant reduction in epididymal weight of reserpine treated rabbit. FSH and LH are hormones released by the anterior pituitary via stimulation of the gonadotropin releasing hormone. While FSH regulates spermatogenesis, LH stimulates the leydig cells which in turn stimulate testosterone release. The result obtained from this study showed no significant difference in serum LH and FSH levels, although there was slight difference but was not significant. Meanwhile it has been reported that chronic treatment of rats with reserpine leads to decrease in hypothalamic secretion of gonadotrophic hormones [23].

Testosterone, the main male androgen, have always been assumed to play a major role in male erectile function as evidenced by the observation that men with marked decrease in testosterone concentration have a significant reduction in the frequency, amplitude and rigidity of erection. Results from this study revealed a significant reduction in testosterone level in reserpine treated animals when compared

with control. This is in agreement with previous study which reported that men with depressive symptoms had lower total testosterone levels than those without depressive symptoms [24]. Ordinarily, low circulating levels of testosterone in men result to decreased energy and libido or often times lead to discomfort, pain and sleep disturbances [25]. Reference [26] also reported that free testosterone levels had a significantly negative correlation with severity of depression.

The observed decrease in testosterone levels obtained in this study was further corroborated with the testicular histological findings that showed leydig cell hyperplasia in reserpine treated group which can consequently alter testosterone synthesis. Histological findings of the corpus carvenosa revealed poor histomorphology while the glans shows loosened connective tissues in corpus spongiosum with a mild lipid deposition and edema.

IV. CONCLUSION AND RECOMMENDATION

Findings from this study revealed that reserpine has serious deleterious effects on the reproductive organs and hormones as observed with the decrease serum testosterone level and epididymal weight. The LH and FSH in circulation appear unaffected. This study therefore suggests that reserpine alters serum testosterone level in male rabbits but the mechanism remains unknown. Further studies are however suggested to unravel the mechanism through which reserpine alters serum testosterone in circulation.

ACKNOWLEDGMENT

The authors acknowledge the astute assistance of all laboratory technologists at the Physiology Laboratory of the College of Health Sciences, Osun State University, during the research.

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