Effect of The Methanolic Extarct of Trichosanthes Cucumerina Seed (Snake Gourd/Tomatoe) on Hormone Influenced Testes Weight of Adult Wistar Rats

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Abstract

Background: To determine the effect caused by the methanolic extract of Trichosanthes cucumerina seed on hormone influenced testis weight in adult Wistar Rats. Place and Duration of Study: Department of Anatomy, Faculty of Basic Medical Science, Olabisi Onabanjo University, Ikenne, Ogun State Nigeria, for 6 weeks. Methodology: We included 20 adult male Wistar rats, of 4 groups (Normal control NC, Hormone treated control HTC, High dose of extract HDT/C, Low dose of extract LDT/C) with each group comprising of 5 animals each, with individual rats weighing between 150g-320g. The testes weight decrease was achieved by the simultaneous induction of 400ng/ml of Estradiol and 1250ng/ml of Testosterone (with respect to the subject’s body weight), which were administered via the inguinal region for a period of 3 weeks (in alternate days). After the period of hormonal administration, two of the subjects from groups NC and HTC were sacrificed in other to appreciate the weight decrease in the HTC group as compared with the NC. Furthermore, with the methanolic extract of the plant’s seed, the animals grouped in HD/TC and LDT/C was treated with respect to their body weight, for a period of 3 weeks. After which, the animals were sacrificed and the testes were further processed. Results: All results were expressed as Mean ± Standard Deviation (S.D) for each group. All grouped data were statistically evaluated using SPSS 15.0 software. Hypothesis testing methods included the independent – samples t–test. Statistical significance was set at p<0.05. Conclusion Steroids taken for muscle enhancement (especially anabolic steroids) often have the undesired side effect of testicular shrinkage and often times affect spermatogenesis

Introduction

The testicle is the male gonad in animals. Like the ovaries to which they are homologous, testes are components of both the reproductive system and the endocrine system. The primary functions of the testes are to produce sperm (spermatogenesis) and to produce androgens, primarily testosterone. Almost all healthy male vertebrates have two testes. They are typically of similar size. In mammals, the testes are often contained within an extension of the abdomen called the scrotum. In mammals with external testes it is most common for one testicle to hang lower than the other. While the size of the testicle varies, it is estimated that 21.9% of men have their higher testicle being their left, while 27.3% of men have reported to have equally positioned testicles.[1] This is due to differences in the vascular anatomical structure on the right and left sides. In healthy European adult humans, average testicular volume is 18 cm³ per testis, with normal size ranging from 12 cm³ to 30 cm³.[2] The average testicle size after puberty measures up to around 2 inches long, 0.8 inches in breadth, and 1.2 inches in height (5 x 2 x 3 cm). The volume is calculated using the formula for the volume of an ellipsoid: 4/3 ? × (length/2) × (width/2) × (depth/2). Human testicles are smaller than chimpanzee testicles but larger than gorilla testicles.[3] Testes contains tunica albuginea, seminiferous tubules, sperm cells, Leydig cells, rete testis, mediastinum testis, efferent ducts, epididymis, and Interstitial connective tissues. Many anatomical features of the adult testis reflect its developmental origin in the abdomen. The layers of tissue enclosing each testicle are derived from the layers of the anterior abdominal wall. Notably, the cremasteric muscle arising from the internal oblique muscle. There are two phases in which the testes grow substantially; namely in embryonic and pubertal age. During mammalian development, the gonads are at first capable of becoming either ovaries or testes.[4] In humans, starting at about week 4 the gonadal rudiments are present within the intermediate mesoderm adjacent to the developing kidneys. At about week 6, sex cords develop within the forming testes. These are made up of early Sertoli cells that surround and nurture the germ cells that migrate into the gonads shortly before sex determination begins. In males, the sex-specific gene SRY that is found on the Y-chromosome initiates sex determination by
downstream regulation of sex-determining factors, (such as GATA4, SOX9 and AMH), which leads to development of the male phenotype, including directing development of the early bipotential gonad down the male path of development. Testes follow the “path of descent” from high in the posterior fetal abdomen to the inguinal ring and beyond to the inguinal canal and into the scrotum. In most cases (97% full-term, 70% preterm), both testes have descended by birth. In most other cases, only one testis fails to descend (cryptorchidism) and that will probably express itself within a year. The testes grow in response to the start of spermatogenesis. Size depends on lytic function, sperm production (amount of spermatogenesis present in testis), interstitial fluid, and Sertoli cell fluid production. After puberty, the volume of the testes can be increased by over 500% as compared to the pre-pubertal size. Testicles are fully descended before one reaches puberty. Testicular size as a proportion of body weight varies widely. In the mammalian kingdom, there is a tendency for testicular size to correspond with multiple mated (e.g., harems, polygamy). Production of testicular output sperm and spermatic fluid is also larger in polygamous animals, possibly a spermatogenic competition for survival. The testes of the right whale are likely to be the largest of any animal, each weighing around 500 kg (1,100 lb).[5] Among the Hominidae, gorillas have little female promiscuity and sperm competition and the testes are small compared to body weight (0.03%). Chimpanzees have high promiscuity and large testes compared to body weight (0.3%). Human testicular size falls between these extremes (0.08%).[6] The paired testicular arteries arise directly from the abdominal aorta and descend through the inguinal canal, while the scrotum and the rest of the external genitalia is supplied by the internal pudendal artery (itself a branch of the iliac artery). The testis has collateral blood supply from 1. the cremasteric artery (a branch of the inferior epigastric artery, which is a branch of the external iliac artery), and 2. the artery to the ductus deferens (a branch of the inferior vesical artery, which is a branch of the iliac artery). Therefore, if the testicular artery is ligated, e.g., during a Fowler-Stevens orchiopexy for a high undescended testis, the testis will usually survive on these other blood supplies. Lymphatic drainage of the testes follows the testicular arteries back to the paraaortic lymph nodes, while lymph from the scrotum drains to the inguinal lymph nodes. To some extent, it is possible to change testicular size. Short of direct injury or subjecting them to adverse conditions, e.g., higher temperature than they are normally accustomed to, they can be shrunk by competing against their intrinsic hormonal function through the use of externally administered steroidal hormones. Steroids taken for muscle enhancement (especially anabolic steroids) often have the undesired side effect of testicular shrinkage. Similarly, stimulation of testicular functions via gonadotropic-like hormones may enlarge their size. Testes may shrink or atrophy during hormone replacement therapy or through chemical castration. In all cases, the loss in testes volume corresponds with a loss of spermatogenesis. Testosterone is a steroid hormone from the androgen group and is found in mammals, reptiles,[7] birds,[8] and other vertebrates. In mammals, testosterone is primarily secreted in the testicles of males and the ovaries of females, although small amounts are also secreted by the adrenal glands. It is the principal male sex hormone and an anabolic steroid. In men, testosterone plays a key role in the development of male reproductive tissues such as the testis and prostate as well as promoting secondary sexual characteristics such as increased muscle, bone mass, and the growth of body hair.[9] In addition, testosterone is essential for health and well-being[10] as well as the prevention of osteoporosis.[11] On average, an adult human male body produces about 7-8 times more testosterone than an adult human female body,[12] but females are more sensitive to the hormone.[13] Testosterone is observed in most vertebrates. Fish make a slightly different form called 11-ketotestosterone.[14] Its counterpart in insects is ecdysone.[15] In general, androgens promote protein synthesis and growth of those tissues with androgen receptors. Testosterone effects can be classified as virilizing and anabolic, though the distinction is somewhat artificial, as many of the effects can be considered both. Anabolic effects include growth of muscle mass and strength, increased bone density and strength, and stimulation of linear growth and bone maturation. Androgenic effects include maturation of the sex organs, particularly the penis and the formation of the scrotum in the fetus, and after birth (usually at puberty) a deepening of the voice, growth of the beard and axillary hair. Many of these fall into the category of male secondary sex characteristics. Estradiol (E2 or 17?-estradiol or Oestradiol) is a sex hormone. Estradiol is abbreviated E2 as it has 2 hydroxyl groups in its molecular structure. Estradiol is about 10 times as potent as estrone and about 80 times as potent as estriol in its estrogenic effect. Except during the early follicular phase of the menstrual cycle, its serum levels are somewhat higher than that of estrone during the reproductive years of the human female. Thus it is the predominant estrogen during reproductive years both...
in terms of absolute serum levels as well as in terms of estrogenic activity. During menopause, estrone is the predominant circulating estrogen and during pregnancy estriol is the predominant circulating estrogen in terms of serum levels. Estradiol is also present in males, being produced as an active metabolite product of testosterone. The serum levels of estradiol in males (14 - 55 pg/mL) are roughly comparable to those of postmenopausal women (< 35 pg/mL). Estradiol, like other steroids, is derived from cholesterol. In plasma, estradiol is largely bound to sex hormone-binding globulin, also to albumin. Only a fraction of 2.21% (± 0.04%) is free and biologically active, the percentage remaining constant throughout the menstrual cycle.[16] In the normal menstrual cycle, estradiol levels measure typically <50 pg/ml at menstruation, rise with follicular development (peak: 200 pg/ml), drop briefly at ovulation, and rise again during the luteal phase for a second peak. At the end of the luteal phase, estradiol levels drop to their menstrual levels unless there is a pregnancy. During pregnancy, estrogen levels, including estradiol, rise steadily toward term. The source of these estrogens is the placenta, which aromatizes prohormones produced in the fetal adrenal gland. Snake gourd (Trichosanthes cucumerina) belongs to the family Cucurbitaceae mostly consumed as vegetable, but it may grow throughout the year except extreme winter. It is a popular vegetable with moderately high nutritive value. The total production of snake gourd during 2003-2004 was 136000 tons on the area of 1, 59, 000 acres of land. [17] This figure indicates the low yield potentiality of our cultivars. It is commonly called as snake gourd, viper gourd, snake tomato or long tomato. The fruit is usually consumed as a vegetable due to its good nutritional value. The plant is richly constituted with a series of chemical constituents like flavonoids, carotenoids, phenolic acids which makes the plant pharmacologically and therapeutically active. Its Fruit is regarded as anthelmintic, vomitive [18] antidia-betic [19] for boil [20]. Seeds are anthelmintic, and anti fibrile [21]. Root is used as purgative and tonic. The pharmacological activities of cucurbitacin containing plants have been known since ancient times. Cucurbitacins are particularly known in folk medicine for their strong purgative, anti-inflammatory, and hepatoprotective activi-ties [22].

Methods

Management Adult male rats, weighing between 150g-320g were obtained from the animal house of the Department of Zoology, University of Ibadan, Nigeria. They were housed in the animal house provided by the Department of Anatomy, Olabisi Onabanjo University, where they had access to food, water, and air. Their environment was well cleaned to avoid infection of any kind upon the animals. After a week of acclimatization, I started inducing them with specific dosage of Estradiol and Testosterone (both diluted in corn oil respectively). The hormones were given based on the animal’s body weight, and the route of administration being the inguinal region. After 3 weeks of induction, some of the subjects were sacrificed to appreciate the testicular shrinkage haven caused by the exogenous hormones. Others were then treated with the methanolic extracts of Trichosanthes cucumerina (in proportion to their body weight), diluted in corn oil. The treatment lasted for 3 weeks after which the animals were sacrificed. Drug Administration The hormones were diluted in corn oil into 400ng/ml and 1250ng/ml of estradiol and testosterone respectively. Hormones were administered exogenously via the inguinal region for three weeks, for thrice a week in alternate days [23]. The extracts were given at 0.2mg/ml and 0.1mg/ml of high and low dosages [23] The Testosterone was manufactured by Green Field Pharm. (Jiang Su) Co., Ltd, China while Estradiol was manufactured by Medipharm (Pvt.) Ltd., 108-Kotlakhpat Industrial Estate, Lahore. Method of Extraction Six ripe fruits of Trichosanthes cucumerina were gotten from Ayepe, a local town in Ijebu-remo side of Ogun State, Nigeria. The seeds were obtained from the fruit, washed in clean water; sun dried for three days, the coat peeled off, 40g of the seeds were weighed, grinded into fine powder and finally soaked in 100ml of methanol. The solution was filtered after 48 hours while the filtrate was concentrated using the rotary evaporator; volume of filtrate obtained ~30.6ml, weight of residue left ~32g; weight of the seeds dissolved in the filtrate ~8g; volume of filtrate (oil obtained from the seeds) after evaporating = 2.5ml. Therefore; extracts concentration = mass/volume = 8/2.5 = 3.2g/ml Processing The animals were anaesthetized with chloroform in closed chamber. The animals were then sacrificed and the scrotum of each rat opened, the testis was ligated and the whole testis was then taken out for the weight to be taken.

Results

1. For the Normal Control group (NCTRL), 5 rats were used and the Mean ± S.D was = 0.30 ± 0.05.
2. For the Hormone treated Control group (HCTRL), 5 rats were used and the Mean ± S.D was = 0.27 ± 0.02.
3. For the High Dose of Plant Extract group (HD T/C), 5 rats were used and the Mean ± S.D was = 0.30 ± 0.04 c, b.

4. For the Low Dose of Plant Extract group (LD T/C), 5 rats were used and the Mean ± S.D was = 0.28 ± 0.04 c, b. b- No significant difference between H CTRL when compared with HD T/C & LD T/C c- No significant difference between N CTRL when compared with HD T/C & LD T/C

Discussion

Using t-test at 5% level of significance, no significant difference (P < 0.05) was observed statistically between the groups (N ctrl and LD T/C). More so, difference between groups (H ctrl and HD T/C) was not statistically significant (P < 0.05). No significance difference (P < 0.05) was observed statistically between the groups (N ctrl and HD T/C). The difference between the groups (H Ctrl and LD T/C) was not statistically significant (P < 0.05). With respect to the table, one would observe reduction in the testes weights (shrinkage) because, no spermatogenic activity occurred, as the spermatogenic cells have been inhibited from their actions, but with the extract administration, the initial spermatogenic state of the testes were approached.

Conclusion(s)

Steroids taken for muscle enhancement (especially anabolic steroids) often have the undesired side effect of testicular shrinkage and often times affect spermatogenesis.

Abbreviation(s)

T/C- Trichosanthis cucumerina
METC- Methanolic extract of Trichosanthes cucumurina seed MEAN ± SD- Mean plus OR minus Standard Deviation p<0.05- Statistical significance was set at p< 0.05. t-test- samples t-test

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