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RESEARCH ARTICLE

Effect Aqueous Extract of (*Cyperus rotundus*) tuber on Histological Structure of Testes in Alloxan-Induced Diabetic Male Albino Rats

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Abstract

Histological structure of testes in alloxan-induced diabetic rats was investigated after daily oral administration of (*Cyperus rotundus*) tuber aqueous extract for one month. Therty were divided into 5experimental groups, each group contain 6 adultmale albino rats weighing 150-200 g. Testes samples were obtained and processed for microscopic evaluation after staining the prepared sections with both Heamatoxylin and Eosin. There was a found severe histopatholoigical changes belong to rats injected with alloxan showing seminiferous tubules completely destroyed and nucleus going out from the testis, also presence many bleeding area, absence the connective tissue and leydig cell while showed No histological changes were found in the examined organs belong to rats treated with Extract of *Cyperus rotundus*.

Keyword: *Diabetes mellitus, Testis, male reproductive system, MSD, Cyperus rotundus, Glimepiride.*

Introduction

Diabetes mellitus is group of metabolic disorders characterised by hyperglycemia, glycosuria and hyperlipaemia. Diabetes was affected approximately 177 million people worldwide in year 2000 and it is expected to increase up to 300 million till year 2025 , it's group of heterogeneous syndromes such as heart attack, stroke and peripheral vascular disease [1].Diabetes are shown to reduce testosterone levels and impair spermatogenesis through damaging testicular functions in experimental animals and humans [2].

Diabetes induced pathological changes in testicular tissues are encountered in tunica albuginea seminiferous tubules and interstitial connective tissue of testes and leydig cells [3].

Both clinical and experimental studies revealed impairment of spermatogenesis, reduced sperm count, sperm motility, seminal fluid volume and low testosterone levels in diabetic subjects[4].Diabetes causes directly or indirectly progressive impairment on Sertoli cells and germ cells, a decrease of testosterone levels and altered spermatogenesis, evidenced by spermatozoa defective. These changes increase with the length of the diabetic state [5].Men with diabetes have been found to have a significantly higher percentage of spermatozoa with nuclear DNA damage [6]. DM induces subtle molecular changes that are important for sperm quality and function [7].

The diabetes effect on primary functions of the male reproductive system are the production of sperm, the transportation of sperm from the testes out of the male body, placement of sperm into the female's vagina and the production of glandular secretions and hormones[8].

It was associated with reproductive impairment in both men and women, About 90% of diabetic patients have disturbances in sexual function, including a decrease in libido, impotence and infertility [9]. DM is an epidemic metabolic disease concurrent with falling fertility rates, which provokes severe detrimental blood testes barrier (BTB) alterations [10].The diabetic patients suffer from an increased risk of oxidative stress-related diseases not only in the present generation but can also transmit the nuclear defects to their progeny [11]. Enhanced oxidative stress and changes in antioxidant

capacity are considered to play an important role in the pathogenesis of chronic DM [12].Moreover, [13] concluded that the neuropathy and vascular insufficiency which caused by diabetes may be related to sexual dysfunction.

Hyperglycemia-induced oxidative stress and associated with testicular failure leading to sexual dysfunction, impotence and infertility. Also oxidative damage the sperm nucleus results in mutation and often these changes in the DNA sequences are transmitted to the offspring, contributing in the heritable disorders in newborns, the changes in the sperm shape and sperm count could be a useful method to study the influence of a disease on the male germinal cells [14, 15].

Testicular and sperm cells have increased susceptibility to free radical damage due to higher level of polyunsaturated fatty acid (PUFA), low oxygen tension and lack of antioxidant defence mechanism [16].Lead to increasing cases of infertility among males [17].Infertile male diabetics tend to suffer from decreased sex libido, diminishing sperm count, endocrine disturbance, impaired penile erection and ejaculation [18; 19].The lipids in the spermatozoa are the main substrates for the ROS mediated peroxidation and damage to it is reported to affect the motility and fertilization capacity of sperms [20].

The oxidative damage initiates sperm plasma membrane destruction, apoptosis and germ cell death [21].The free radicals induce genotoxicity by initiating sperm DNA denaturation and fragmentation with limited DNA repair. Damage of genetic material in spermatogonia and spermatocytes has been related to the increased sperm abnormalities. These alterations bring about possibilities of genetic disorders if passed down to offspring [22].There are three main dysfunctional mechanisms may be postulated to explain the sperm damage (MSD) observed in patients with diabetes: endocrine disorders, diabetic neuropathy, and oxidative stress. [23].

DM causes impairment of reproductive activity and leads to infertility [24]. Diabetic rats have been shown to possess low serum testosterone with decreased testicular weight, sperm count and sperm motility [25].Diabetes is usually accompanied by increased production of free radicals or impaired antioxidant defense [26].

Mitochondria are the principal source of ROS in cells and impairment of mitochondrial function is intrinsically related to diabetes [27].In diabetic men and animal models indicate that DM causes male infertility based on impotency, retrograde ejaculation and hypogonadism. DM effect on sperm quality, altered spermatogenesis, morphological changes in testes, altered glucose metabolism in Sertoli-blood testes barrier, reduced testosterone, ejaculatory dysfunction and reduced libido [7,10].

The molecular mechanism responsible for the alterations induced by DM in male reproductive potential including endocrine disorders, neuropathy and increased oxidative stress [23]. DM induced adverse effects on male reproductive functions through hormonal alterations in the hypothalamicpituitarygonadal axis or through the direct interactions of insulin with the testes and sperm cells, as both the testes and sperms themselves produce insulin [28].

Two mechanisms were caused alterations in Leydig cells: (1) the effect of hypoinsulinaemia on serum LH level (2) the combined effects of increased level of LH and insulin on Leydig cells [29]. The alter activity of arginase (an enzyme important in cell proliferation) in the reproductive system, which may impair Leydig cell proliferation [30].

The medicinal plants are useful for this study is *C.rotundus* L., (Family-Cyperaceae) also known as purple nut sedge or nut grass, is a common perennial weed with slender, scaly creeping rhizomes, bulbous at the base and arising singly from the tubers which are about 1-3 cm long. The tubers are externally blackish in colour and reddish white inside, with a characteristic odour. The stems grow to about 25 cm tall and the leaves are linear, dark green and grooved on the upper surface. Inflorescences are small, with 2-4 bracts, consisting of tiny flowers with a red-brown husk.

The nut is three-angled, oblong-ovate, yellow in colour and black when ripe. *C.rotundus* is indigenous to India, but is now found in tropical, subtropical and temperate regions [31]. Phytochemical studies have shown that the major chemical components of this herb are essential oils, flavonoids, terpenoids,

mono-and sesquiterpenes The plant contains the following chemical constituents- Cyprotene, cypera-2, 4-diene, a-copaene, cyperene, aselinene, rotundene, valencene, ylanga-2, 4- diene, g-gurjunene, trans-calamenene, d-cadinene[32].Healing as well as for curing of human diseases because of the presence of phytochemical constituents.

Phytochemicals are naturally occurring in the medicinal plants, leaves, vegetables and roots that have defense mechanism and protect from various diseases, Phytochemicals are primary and secondary compounds [33].

[34]

Medicinal Uses

Anti-Inflammatory Activity, Antipyretic activity, Analgesic activity, Tranqulizing activity, Hypolipidaemic Activity, Hepatoprotective activity, Anti-obesity activity, Antimicrobial activity, Wound healing activity, Antioxidant activity, Anticancer activity, Antidiabetic activity, Anti-Platelet Activity, Anti-Candida activity [35].

Material and Method:

*Cyperus rotundus*rhizome Extract Aqueous Preparation

Cyperus Rotundus Rhizome plant was collected in southalnajafal-ashraf. Rhizome plant waswashed well with water dried at room temperature in the dark and then ground in anelectric grinder to obtain a coarse powder. Then50 g of the plant powder was suspended in 500mL distilled water, The decoction obtained was filtered by Whatman filter paper No.1 Then extracts were then evaporated at 45˚C by rotary evaporator to form a paste. Then stored at-20°C until used [36].

Histological Study

Animals were doping by chloroform and cutting the testes tissues taken from experimental animals were fixed in 10% neutral formalin, then transferred to a series of ethanol alcohol-dehydrated(70,80,90,100,100%) for 1.30 hour then cleared in xylene for two time ,the tissues were infiltrated in molten paraffin wax in the oven at 58°C. Three changes of molten paraffin wax at one-hour intervals were made, after which the tissues were embedded in wax and blocked out. Prior to embedding, it was ensured that the mounted sections to be cut by the rotary microtome were orientate, paraffin-embedded and the section to mean thickness of 5 µm. The histological examination was evaluated by assessing the morphological changes with Hematoxylin and Eosin (H&E) stains techniques, after which they were passed through ascending grade of alcohol, cleared in xylene and mount in DPX mount ant, allowed to dry at room temperature and observed Histopathologically under digital light microscope [37].

Induction of Diabetes

Alloxan is a urea derivative which causes selective necrosis of the ß- cells of pancreatic islets. In addition, it has been widely used to produce experimental diabetes in animals such as rabbits, rats, mice and dogs with different grades of disease severity by varying the dose of alloxan used [38, 39].

Diabetes was induced in fasting rats 12hrs by a single intraperitoneal dose of 150 mg/kg b.w. of alloxan dissolved in0·9% saline, and the diabetic state was assessed by measuring the fasting plasma glucose concentration 72 hrs after alloxan treatment in fasting rats, and the rats with a plasma glucose level

above 250 mg/dl were selected for the experiment and considered as diabetic [40].

Experimental Design

Experimental (30) rats aged three months were divided into five groups of six animals each and treated for 30 days as follows:

Group A - Normal control (administration normal salin)

Group B - Diabetic control (injected with alloxan)

Group C - Diabetic+ Amyral 1mg/kg b.wt)

Group D - Diabetic+hot aqueous Extract of *Cyperus rotundus* tuber (750 mg/kg b.wt)

Group E- only hot aqueous Extract of *Cyperus rotundus* tuber (750 mg/kg b.wt)

Result and Discussion

The results show significantly decreased seminiferous tubule diameter, increased testicular blood vessel numbers, and altered tubule stage distribution compared with controls, various mechanisms may explain the sperm damage observed in patients with DM. [23].These include endocrine disorders, neuropathy, and increased oxidative stress. Many authors' suggest that DM decreases serum testosterone levels.

This is associated with a steroidogenetic defect in Leydig cells. In addition diabetic neuropathy seems to cause atonia of seminal vesicles, bladder, and urethra. Furthermore, DM is associated with an increased oxidative stress, which damages sperm nuclear and mitochondrial DNA. Finally, spermatogenesis derangement and germ cell apoptosis in type 1 DM may relate to a local autoimmune damage, whereas insulin resistance, obesity, and other related co morbidities may impair sperm parameters and decrease testosterone serum levels in patients with type 2 DM [23].

The present review describes the ameliorative effects of medicinal plants or their products, especially on male reproductive dysfunctions, in experimental diabetic animal models. medicinal plants have been used for the management of the diabetes mellitus in various traditional system of medicine and in folklore worldwide as they are a rich source of bioactive phytoconstituents, which lower blood glucose level and/or also act as antioxidants resulting in the amelioration of oxidative-stress-induced diabetic complications [41].*Cyperus rotundus* that contain high mount of antioxidant that treatment the infertility [42].

Glimepiride is a third-generation antidiabetic sulphonylurea known to have antioxidant effect in STZ-induced diabetes. The administration of glimepiride decreased sperm shape abnormalities, enhanced sperm counts, and improved antioxidant status in the diabetic rats, glimepiride inhibits sperm abnormalities and enhances the antioxidant defence in diabetic rats [43]

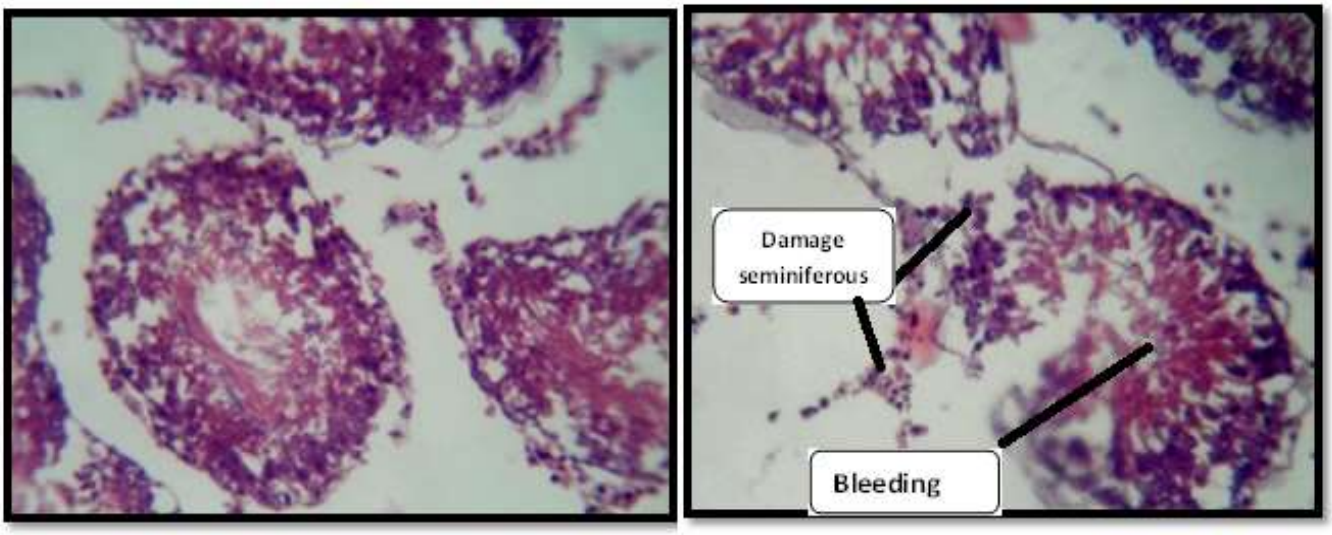

Fig. 1: Section in testes tissue belongs to diabetic rat showingseminiferous tubules completely destroyed and nucleus going out from the testis, also presence many bleeding area ,absence the connective tissue and leydig cell (H&E 40x)

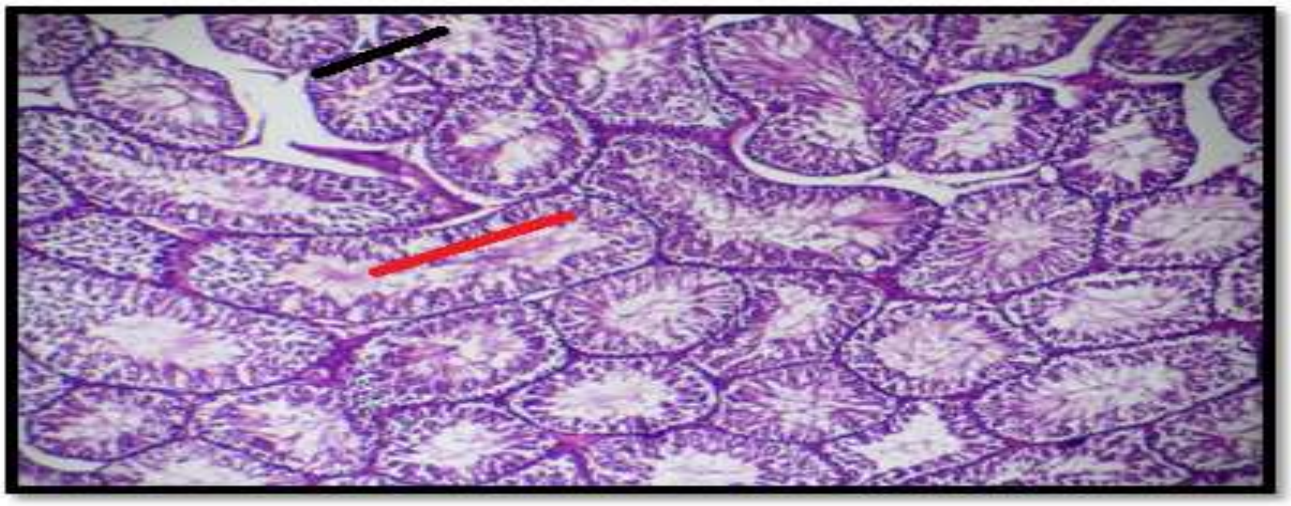

Fig. 2: Section in testes tissue belongs to rat treated with normal salin (nomal control showing arranged seminiferous tubules, presence connective tissue that contain leydig cell, interstitial matter (H&E 10x)

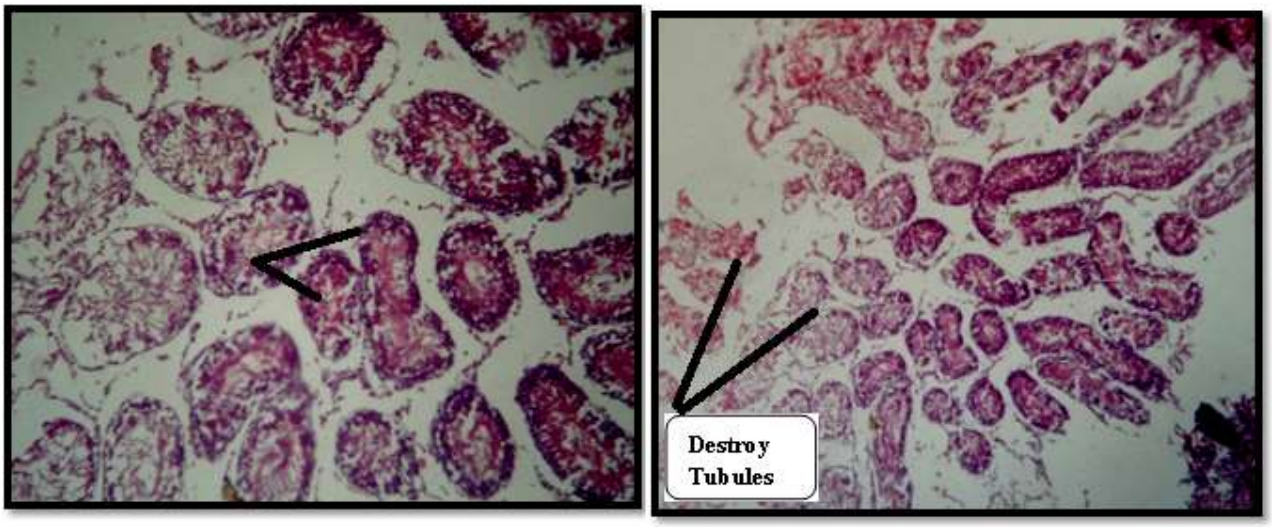

Fig. 3: Section in kidney tissue belongs to diabetic rat treated with Amyralseminiferous tubules completely destroyed and nucleus going out from the testis, also presence many bleeding area ,absence the connective tissue and leydig cell (H&E 40x and 10 x)

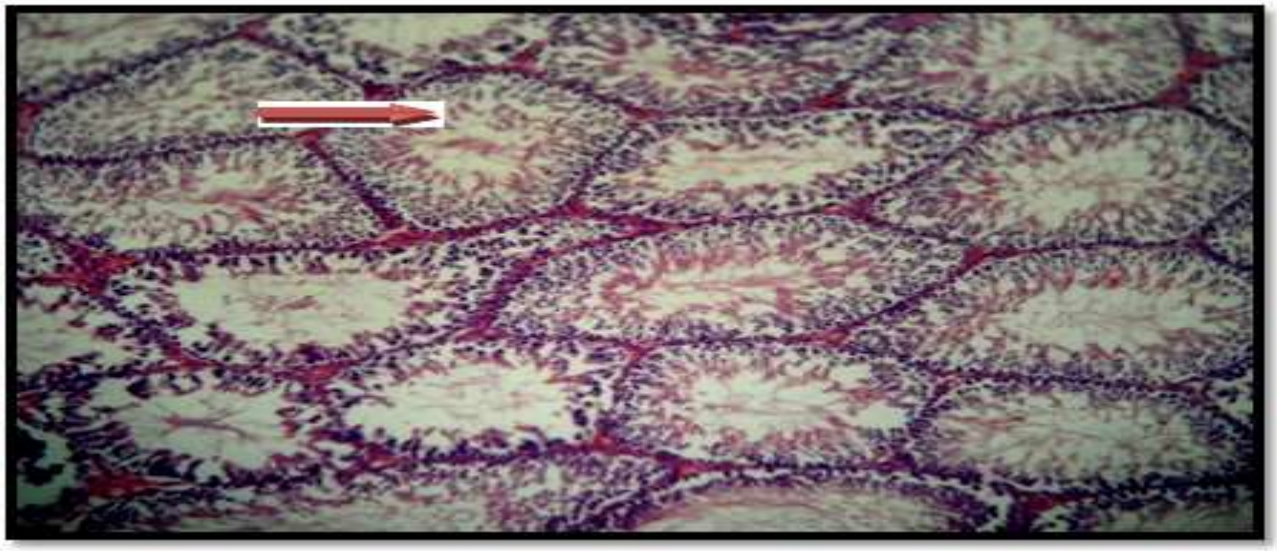

Fig. 4: Section in testes tissue belongs to rat treated with Cyperus rotundus extract showingarranged seminiferous tubules,presence connective tissue that contain leydig cell , (H&E 40x)

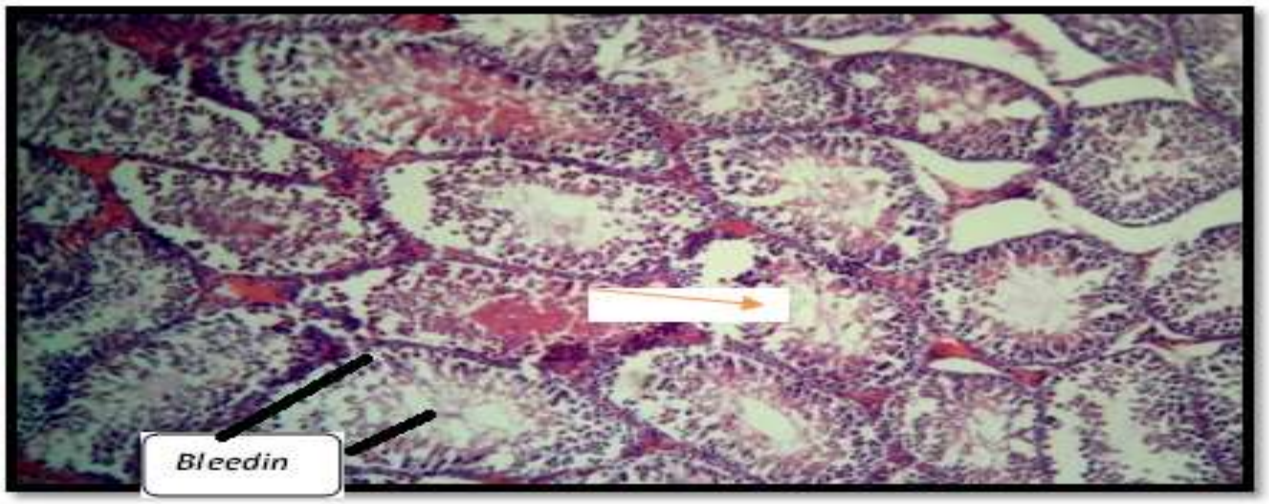

Fig. 5: Section in testes tissue belongs to diabetic rat treated with Cyperus rotundus extractand Amyral showingarranged seminiferous tubules, presence connective tissue that contain leydig cell, (H&E 40x)

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