Effects of Morus alba leaves extracts on sperm count and testicular weight in experimentally streptozotocin induced diabetes male rats.

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Abstract

This study aims to investigate the effects of Morus alba leaves extracts (ethanol hydro-alcohol 60%) on (**sperm count and testicular weight**) in experimentally streptozotocin induced diabetes in male rats. Fourty adult male albino rats weighting (150 -200 g) were used and divided into 4 experimental groups, 10 rats in each group: The first group was served as control group. The remaining groups were injected intra pretonial by streptozotocin (STZ) at 45 mg/kg b.wt to induce diabetes. The second diabetic group was received as control diabetic group. The third diabetic group was treated with Cidophage (500 mg/kg, orally).While, the fourth diabetic groups were treated with Morus alba leaves extracts (600 mg/kg b.w orally). All treatment were given daily for successive 30 days. After end treatments all rats were sacrificed and parameters were measured. The obtained results demonstrated the use of Morus alba leaves extracts improve of sperm count and testicular weight of diabetic rats. Hydro-alcoholic extract 60% of Morus alba leave could improve the **sperm count and testicular weight** of diabetes male rats.

Keywords: medicinal plants, sperm count, testicular weight, Morus alba leave extracts.

Introduction:-

Diabetes is a chronic disease characterized by high levels in blood glucose and abnormal metabolism of carbohydrates, proteins, and fat associated with a relative or absolute insufficiency of insulin secretion and with numerous degrees of insulin resistance. Such alterations result in increased blood glucose, which causes long-term complications in many organs[1]. Despite important progress in the management of diabetes using synthetic drugs, many traditional plant treatments are still being used throughout the world. Plants are valued in indigenous systems of medicine for the treatment of various diseases [2]. Medicinal plants provide a good source of oral hypoglycemic compounds for the development of new pharmaceutical leads in addition to dietary supplements to existing therapies [3]. Some of the plants that are being used for the treatment of diabetes have received scientific or medicinal scrutiny and even the World Health Organization's expert committee on diabetes recommends that this area warrants further attention [4].

Leaves and shoots from the mulberry tree possess several medicinal properties,including hypoglycemic, hypotensive, and diuretic effects[5]. Mulberry root bark or leave extracts were shown to possess hypoglycemic effects in animal models [6].

The extract of Morus alba leaves promoted significant hypolipidemic activity in experimental animals [7].

Anthocyanin components from Morus alba fruits were isolated and identified through [8] to study their antioxidant effect. The authors reported that, cyanidin 3-rutinoside and cyanidin 3-glucoside are of valuable importance as antioxidants, Mulberroside A is the major stilbene glycoside of Morus alba and it showed inhibitory effect against $FeSO_4/H_2O_2$ induced lipid peroxidation in microsomes of rat, also found that the Mulberroside A have scavenging effects on DPPH (1, 1-diphenyl-2-picrylhydrazyl) radical [9].

The Mulberry fruits increase the strength of the antioxidant protecting system and diminish the damaging of the oxidative substances in red blood cells (RBCs) of the experimentally induced diabetes in rats **[10]**. The aim of the present study was to clarify the effect of Morus alba leave extracts on **Sperm count and testicular weight**.

Material and Methods

1- Materials

Streptozotocin (STZ) was purchased from Sigma Company (USA), Cidophage was obtained from CID Company (Egypt), NaCl 0.9%, sodium citrate, citric acid, ethyl alcohol. 95% were purchased from El-Gomhoria Company.

Morus alba leave extracts :-

Morus alba Leaves were collected, cleaned, washed with tap water, dried and stored in dry atmosphere. The alcoholic extract of Morus alba leaves was suspended in distilled water according to the method of [11] by the use of soxhlet apparatus, and orally administrated of Morus alba leave 600 mg/kg b.wt [12], by stomach tube daily for 30 days.

Cidophage (Metformin hydrochloride 500mg) CID Company (CID, Giza, Egypt) and it was administrated orally by stomach tube in a dose 500 mg/kg b.w [13].

Induction of diabetes:

Induction of diabetes was done by using streptozotocin (STZ) at 45mg/kg b.wt in rats according to [14].

Experimental Animals:

A total of fourty (**40**) adult healthy males rats with age ranged between 8-10 weeks, and their weight

ranged between (150-200) grams, were used in this study. Animals were left for one week to acclimatize the place. Animals were kept in cage in a controlled environment, maintained under a 20-25°C and light period of 12 hours daily and (50-70)% humidity. Rats provided with standard diet and water ad-libitum. The animals were housed in plastic cage. Care was taken to avoid any unnecessary stress. The cages were cleaned twice a week.

Experimental design:-

After one week period of acclimatization in cages condition, rats were divided into 4 groups (each of 10 rats) as follows :

Group I: (control clinically healthy)treated with 0.2 ml distilled water orally.

Group II: diabetic non -treated (45 mg/ kg b.wt. STZ) intra peritonea [14].

Group III: diabetic treated with 500 mg/kg b.wt. Cidophage orally / day by stomach tube for 30 days [13].

Group IV: diabetic treated with 600mg/kg b.wt. Morus alba leaves extract orally daily for 30 days [12].

Sampling:-

Preparation of epididymal tail sperm suspension.

After the end of the experiment, animals were weighed by a sensitive balance then anesthetized by diethyl ether.

Abdominal cavity was opened, testes and epididymus excised and soaked in physiological normal saline and cleared from attached fat and connective tissue. Testis were weighed by a sensitive balance. The tail of the left epididymus was taken and immersed in one ml of physiological normal saline at 37°C in a watch glass, then the tail was cut by microsurgical scissors, to perform the following examination on sperm characters [15].

2- Methods

A- Determination of Sperm concentration.

Sperm count was done according to [15]. By using Hemocytometer (Neubauer Type).

The Hemocytometer sides were filled with 5µl of a sperm suspension and covered by cover slide; the sperms were counted in twenty-five small squares of the chamber. Estimation of sperm was made according to the following formula:

Sperm concentration = Number of sperm X 10000

B- Determination of testicular weight to body weight ratio: -

After treating period, animals were weighed, anesthetized by diethyl ether. Testis were removed and weighed by sensitive balance after being cleaned from the accessory connective and adipose tissues. Testicular weight to body weight ratio was calculated as in the following equation:

Testicular wt-to-body wt ratio = (Wt. of testis (gm) / Wt. of animal (gm) X 100.

Statistical Analysis:

Data were subjected to statistical analysis using statistical software program (SPSS for Windows, version 18, USA). Means and standard error for each variable were estimated. Differences between means of different groups were carried out using one way ANOVA with Duncan multiple comparison tests. Dissimilar superscript letters in the same column show a significance ($P<0.05$) [16].

Results

1. Effect of Morus alba leaves extracts on sperm counts:-

It was observed clearly from table (1) that sperm counts was significantly decreased ($P<0.05$) in diabetic group (1375000 ± 72168) in comparison with the control group (2375000± 505799) after treatment. Meanwhile sperm counts was significantly increased ($P<0.05$) in diabetic treated groups with 1987500 ± 408439 (Cidophage) 3362500 ± 262500 (Morus alba leave hydro-alcoholic extract) in comparison with control diabetic group.

2. Effect of Morus alba leaves extracts on Testicular weight:-

It was observed clearly from Table (1) that Testicular weight was significantly decreased ($P<0.05$) in diabetic group (0.4340 ± 0.6) in comparison with the control group (0.7760±0.4) after treatment. Meanwhile Testicular weight was significantly increased ($P<0.05$) in diabetic treated groups with 0.4230 ± 0.10 (Cidophage) 0.6183± 0.06 (Morus alba leave hydro-alcoholic extract) in comparison with control diabetic group.

Table (1): Determination of serum Sperm count and Testicular weight in diabetes and non diabetic rats. (Mean ± SE) (n=10)

No.	parameters / Group	Sperm count (gm)	Testicular weight
1-	G1 (Control given 0.2ml normal saline)	2375000± 505799	0.7760 ± 0.4
2-	G2 (Diabetic by 45 mg/kg b. wt. STZ)	1375000 ± 72168	0.4340 ± 0.6
3-	G3 (Diabetic treated with Cidophage at 500 mg/kg b. wt.)	1987500 ± 408439	0.4230 ± 0.10
4-	G5 (Diabetic treated with alcoholic extract of Morus alba leaves at 600 mg/kg b. wt.)	3362500 ± 262500	0.6183 ± 0.06

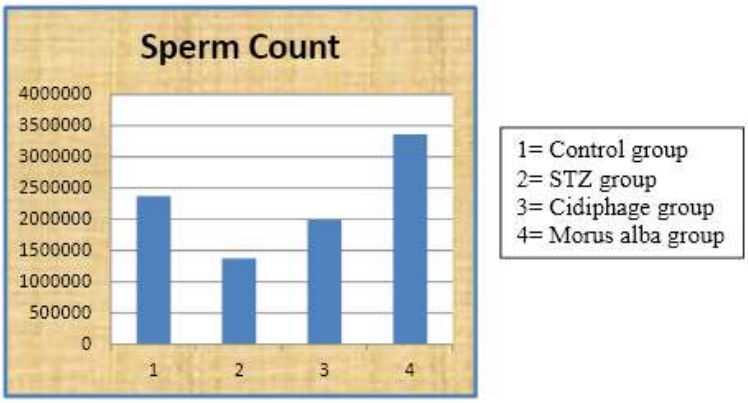

Fig. (1): Determination of sperm count in diabetes and non diabetic rats. (Mean ± SE) (n=10).

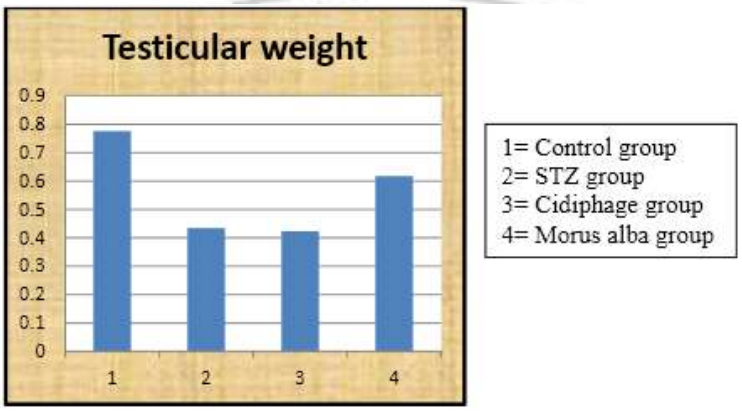

Fig. (2): Determination of testicular weight in diabetes and non diabetic rats. (Mean ± SE) (n=10).

Discoussion

- Effects of Morus alba leaves extracts on Sperm count and Testicular weight:-

In the present study result recorded significant increse in sperm count and testicular weight of all treated groups in comparing with STZ untreated negative control. This finding is supported with the data obtained by [17] explained that the Morus alba is rich in polyphenolic compounds especially the flavonoids and among the flavonoids quercetin 3-(6-malonylglucoside) is most significant for antioxidant potential of mulberry plant that lead to improving effects. The Morus alba leaves containing higher amount of quercetin which is responsible for reduction of oxidation process in vivo and in vitro[18].

The ethanolic extract of Morus alba leaves contains oxyresveratrol and 5,7-dihydroxycoumarin 7-methyl ether which scavenge superoxide and have antioxidant potential effects [19]. Mulberroside A is a major stilbene glycoside of Morus alba and It showed inhibitory effects against FeSO4/H2O2-induced lipid per oxidation in microsomes of rat and also found that Mulberroside A have scavenging effect on DPPH (1,1-diphenyl-2-picrylhydrazyl) radical [9]. The anthocyanin is present in mulberry extract and it is a natural colorant constituent for the plant, anthocyanins showed antioxidant activity by scavenging the peroxyl radicals in trapping reaction[20]. Mulberry plants contains many active compounds which acts as an antioxidant like polyphenols, carotenoids and vitamin A, C, E. They found that these compounds increase the body's antioxidant status and regulate Low-density lipoprotein (LDL) oxidation through different mechanisms [21]

A complication of chronic Diabetes mellitus causes the decreasing of LH, FSH and testosterone levels [22]. FSH, LH and testosterone has an important role in spermatogenesis process [23]. If the amount of these hormones reduces, it will disturb the process of spermatogenesis, and the final consequency will be followed by the decreasing of germ cell numbers as well as testicular weight [24].

Approximately fifteen polyhydroxylated alkaloids have been isolated from the leaves of mulberry, one of which is 1-Deoxynojirimycin (DNJ), which has potency to decrease blood glucose by inhibiting alpha-glucosidase [23]. This enzyme catalyzes the hydrolysis of bonds in maltose to produce two molecules of glucose [25]. Leaves and roots extract of mulberry contain 0.24% DNJ compounds [26]. Mulberry leaves also contain several chemical compounds such as ecdysterone [27] and scopoletin [26] which also contribute for the decreasing of blood glucose. In addition, mulberry leaves also contain folic acid and zinc that are able to increase the number of sperm cells in men with infertility experience [27].

mulberry leaves are able to repair tissue damage due to their antioxidant content. One of antioxidant content of mulberry leaves is vitamin C [28]. The role of ascorbic acid (vitamin C) for diabetes is as aldose reductase enzyme inhibitor [25]. thus reducing the use of equivalent reduced. The willingness of the reducing equivalent is useful for the conversion of oxidized glutathione disulfide (GSSG) to reduced glutathione (GSH). It can further prevent the buildup of sorbitol in tissues [29].

The increase in weight of testes of treated group with Morus alba compared to STZ group this happens because the number of spermatogenic cells in the testes also increased. This is consistent with the statement of [23] that the rich content of spermatogenic cells in the seminiferous tubules in the testes can also increase the weight of the testis itself although testicular weight was also influenced by other factors. Testes weight is not only influenced by the presence of germ cells and seminiferous tubule diameter, testis weight was also affected by connective tissue and smooth muscle cells [27]. This network serves to support the process of spermatogenesis is done by the testes. So not only gained weight testicular tubules but also supported by a network of connective tissue and blood vessels. The increasing of the testes weight in diabetic rats after mulberry leaves infusion is due to folic acid and zinc in mulberry leaves that finally support the increasing the number of spermatogenic cells [24].

Conclusion

The obtained results showed the use of Morus alba leaves extracts improved of sperm count and testicular of diabetic rats. Moreover, Morus alba leaves extracts are capable of improving the impaired above parameters and minimized the side effects of STZ diabetic animals.

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تاثير مستخلص اوراق التوت على عدد النطف ووزن الخصى في ذكور الجرذان المصابة تجريبيا بالسكري

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الملخص

الهدف من هذه الدراسة: تهدف هذه الدراسة الى معرفة تأثير كل من مستخلص أوراق التوت (ethanolic hydro-alcohol 60 %) على عدد النطف و وزن الخصى في ذكور الجرذان المصابة تجريبيا بداء السكري باستعمال مادة ستربتوزوتيسين (STZ).

أستخدم في هذة التجربة اربعين من ذكور الجرذان البيض وزنها يتراوح مابين (١٥٠-٢٠٠غم) وتم تقسيمها إلى اربع مجاميع (لكل مجموعة ١٠جرذان) : تركت المجموعة الأولى كمجموعة سيطرة. تم حقن المجاميع المتبقية (بالغشاء البريتوني) بمادة ستربتوزوتيسين (STZ) بجرعة ٤٥ ملغم / كغم من وزن الجسم لإحداث مرض السكري. المجموعة الثانية اعتبرت مجموعة سيطرة ومصابة بالسكري. عولجت مجموعة السكري الثالثة بدواء السيدوفاج (٥٠٠ ملغم / كغم، فمويا). بينما تم علاج المجموعة الرابعة مستخلص ورق التوت بجرعة ٦٠٠ ملغم / كغم من وزن الجسم. استمرت فترت العلاج يوميا لمدة ٣٠ يوما متتالية.

بعد نهاية فترة العلاج تم قتل جميع حيوانات التجربة وتم قياس وزن الخصى وعدد الحيامن.

النتائج : أظهرت النتائج أن استخدام مستخلص ورق التوت آدى إلى تحسين واضح في وزن الخصى وعدد الحيامن.

الاستنتاجات: المستخلص المائي الكحولية لاوراق التوت آدى إلى تحسين في وزن الخصى وعدد الحيامن في ذكور الجرذان.