EFFECT OF *Citrullus colocynthin* ON CERTAIN SPERM FUNCTIONS AND LIVE BIRTH IN MICE: EXPERIMENTAL MODEL FOR MAMMALS

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Abstract

The goal of this study is to find out the effect of *Citrullus colocynthin*(CC) on sperm function parameters and live birth in mice as a model for mammals. Balb/C male and female mature mice , were used in three experiments. Experiment No.1, the male mice were randomly divided into five groups each contains 6 animals according to different concentrations of CC(2.4,4.8,7.2 and 9.6mg/kg/day) and control group. Experiment No. 2,twenty four females were divided into four groups (6 females in each group)untreated and treated with 1.2 ,4.8, and 7.2 mg/kg/day of CC. Experiment No.3,Four mature male mice were coupled with eight mature females, both were treated only with 4.8mg/Kg/day, and the number of live births were recorded .All the mice groups were treated with CC orally for eight weeks. There was a significant ($P<0.05$) decrease in the mean of sperm concentration of mice treated with 7.2mg and 9.6 mg /kg/day of CC compared to control, 2.4mg and 4.8 mg/kg/day of mice groups. Percentage of sperm motility and grade activity of progressive movement of mice treated with 4.8 mg/kg/day was significantly ($P<0.05$) higher than that of other CC concentrations. A highly significant ($P<0.01$) reduction was observed in the percentage of morphologically abnormal sperm(MAS) following treatment with 4.8mg of CC compared to other groups . There was a significant ($P<0.05$) increase in the number of live birth in treated male and female group (9.0 ± 0.4) compared to untreated group(6 ± 0.3). The present study revealed that CC in limited doses can be used for *invivo* improvement of certain sperm characters and to enhance the fertilization ability of the females.

Introduction

Recently ,Many human health centers believed in medical herbs as effective medicine for different diseases. One of these medical herbs is *Citrullus colocynthin* (CC).This plant is belong to *Cucurbitaceae* family which consists of nearly 100 genera and over 750 species [1].Physicians desk reference(PDR®) for herbal medicine [2] observed that the most important parts of CC is the seeds and bitter apples that removed from the harder outer layer of the plant. The seeds extracts have an insulintropic effect which could at least partially account for the antidiabetic activation of the fruits [3].Preparations of bitter apples are used exclusively in fixed combination in the treatment of acute and chronic constipation with various causes as well as in pregnancy. Cucurbits are well recognized source of secondary metabolites ,alkaloids and abortifacient proteins[4,5,6]. Trichosanthin ,other component of CC, is of particular interest because its ribosome- inhibiting properties have shown to be effective in inhibiting the replication of human immunodeficiency virus (HIV) in infected lymphocyte and phagocyte cells, indicating potential as a therapeutic agents for AIDS[

7].Moreover, CC may be advised for ovarian trouble and during menstruation, if the pain griping spasmodic, sharp and severe [8].Although, investigations had been proved the effect of CC on female reproductive system ,no literatures were found to concern on the plant effects on male reproductive system. Therefore ,the purpose of this study is to elucidate the effect of CC on certain sperm functions characters and in turn the fertilization ability of treated males through natural mating with natural and treated females to find out the number of live birth in mice, as experimental model for mammals.

Materials and Methods

-Management

Balb/C mature mice ,8-10 weeks old were used for this study. Diet were freely available for the animals . The mice were placed in an air conditioned room at 22-25C° with light period of 13± 2 hours.

-Preparation of CC :The bitter apples of CC obtained from Iraqi medical herbs pharmacy, were crushed to obtain a powder like material. One gm of CC was dissolved with one litter of distilled water as stock solution.

Experiment No. 1:

The male mice were randomly divided into five groups each contains 6 animals according to different concentrations of CC as follows:

group1 received normal saline ,the control. Group2 ,3 4 and 5 treated for 8 weeks with 2.4 ,4.8 ,7.2 and 9.6mg/kg/day of CC extract ,respectively. All groups were orally drinking the different concentrations of prepared CC.

Male mice were sacrificed by cervical dislocation .Caudal epididymal region was isolated and minced .Sperm function characters were recorded following direct activation technique as described by Al-Dujaily[9].

Experiment No. 2

twenty four female mature Balb/C mice were divided into four groups (6 females in each group) and treated with 1.2, 4.8, and 7.2mg/kg/day of CC. The administration of CC solution was continued for 8 weeks .Then following mating with fertilized male mice, Live birth was recorded.

Experiment No.3

Four mature male mice and eight mature females were treated orally with 4.8 mg/Kg/day of CC extract for eight weeks and mated with each others at the end of treatment. The live births were recorded and compared with live births born from mating of untreated females and males as control group(4 untreated females).

Statistical analysis

Statistical analysis

Statistical analysis was done using F-test for mice treated with different concentrations of CC. The differences were compared by LSD .The live birth results were analyzed by Chi-square($\chi 2$). When P reach 0.05 value ,the result considered significant [10].

Results

Effect of administration of different concentration of CC on certain sperm function of male mice was shown in table 1.There was a significant ($P<0.05$) decrease in the mean of sperm concentration of mice group treated 7.2mg and 9.6mg /kg/day of CC compared to control, 2.4 mg and 4.8 mg/kg/day groups .Percentage of sperm motility was significantly($P<0.05$) higher in the control , 2.4 mg and 4.8 mg groups compared to

7.2mg and 9.6mg groups. Sperm grade activity of progressive movement of mice treated with 4.8 mg/kg/day was significantly ($P<0.05$) higher than that of other CC concentrations. A highly significant ($P<0.01$) reduction in the percentage of morphologically abnormal sperm(MAS) was observed following treatment with 4.8 mg of CC compared with other groups .At the same time, the MAS of the 9.6mg group was significantly($P<0.05$) higher than that of other groups (table 1)

Table 2 showed, the live birth following administration of CC to male and female mice. There was a significant($P<0.05$) increase in the number of live birth in treated male mice group (9.0±0.4) compared to untreated male(6±0.3).The number of live birth in the groups treated with 4.8mg of CC was revealed a significant($P<0.05$) improvement compared to other groups. The number of live birth mice following administration of 7.2 mg to females showed a significant ($P<0.05$) decrease in comparison to other mice groups.

Discussion

In this investigation ,it has been found that CC can be used to enhance sperm function parameters and increased live birth outcome when the mice drink this herb for two months. The results of the present study was not comparable with other studies which believed that CC acts as a harmful substance for fertility potential [11] and may be toxic and can cause diarrhea ,anemia [12] with hepatotoxicity especially haematological changes[13] .However, all these studies were used a high doses of CC than that used in our study .It has been found that CC stimulated H_2O_2 formation[13]. The production of free radicals in high amounts will induced lipid peroxidation leading to oxidative stress and damage to the cell and that may cause apoptosis[14] and pathological changes. Whereas, generation of ROS in **small quantities is fundamental** to sperm capacitation and hyperactivity motility and promote acrosome reaction [15].Thus ,the low dose of CC may be contributed to the improvement of sperm function characters through small production of ROS. The study also indicated an increase in the live birth of mice treated with 4.8 mg/Kg/day of CC for two months. This finding may be resulted from the constituents of CC .The CC contains a number of different amounts of fatty acids namely ; palmitic ,stearic, oleic and linoleic [16].These fatty acids may play an important role in oocyte maturation, fertilization and preimplantation embryos. It has been recorded that the fatty acids found in different concentration but the same number in the oocytes ,ovarian follicular ,oviductal and uterine fluids [17]. However, increase the CC concentration to 7.2mg/Kg/day result in a decrease in the number of live birth, which revealed the maximum limit of CC(4.8mg/Kg/day) that positively effect the reproductive system and the point which negatively response to CC action. Therefore, it is concluded from the present study that CC can be used for the improvement of certain sperm characters of males and to enhance the fertilization ability of the females. further study is recommended to find out its effect on follicular growth and ovulation process.

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Table 1:Effect of CC on certain sperm function parameters in mice following 15 minutes incubation in vitro

Sperm function parameters	CC concentration (mg/Kg/day)				
	Control	2.4mg	4.8 mg	7.2mg	9.6mg
Sperm concentration x10/ml	50±4.5	52.6±6.7	45.5± 6.8	33.5±5.5*	30±7.1*
Sperm motility(%)	+ 60±3.3	* 50±4.6	+ 60±4.8	45±3.8	40±6.2
Grade activity(degree)	2.7±0.3	2.5±0.4	* 3.0±0.2	2.0±0.4	2.3±0.4
Morphologically abnormal sperm (%)	40±8.6	38±7.8	+ 26±7.5	40±6.9	* 50±7.4

Values are mean ± SEM
No. mice per group=6
*P< 0.05 significantly different from other concentrations.
+P<0.01 significantly different from other concentrations.

Table 2:Live birth following administration of CC to male and female mice for 8 weeks

mice groups	No. live birth following CC Administration			
	control	1.2mg/Kg /day	4.8mg/Kg/ day	7.2mg/Kg/ day
1-treated females only+	5.6±0.3	5.5±0.6	7.0±0.4	3.8±0.65*
2-treated males and females	6±0.3 n =4	---	* 9± 0.4 n=8	---

Chi-square
+ No. females per group=6
*P<0.05 : significantly different from other group(s)
++P<0.05: significantly different from 6 mg group

الخلاصة

أجريت الدراسة بهدف دراسة تاثير نبات الحنظل (*Citrullus colocynthin* CC) على معايير وظائف النطف الرئيسية والولادات الحية في الفئران كموديل تجريبي للبائن . أستخدمت ذكور واناث ناضجة لفئران من نوع Balb/Cفي ثلاث تجارب. التجربة الأولى، قسمت ذكور الفئران عشوائيا الى خمسة مجاميع تتكون من ٦ ذكور طبقا للتراكيز المختلفة من نبات الحنظل (2.4 ، 4.8، 7.2، و 9.6 ملغم/ كغم/ اليوم) أضافة الى مجموعة سيطرة . التجربة الثانية قسمت اربعة وعشرون انثى الفار الى اربعة مجاميع (ستة في كل مجموعة) وعولجت بتراكيز 1.2، 4.8، و 7.2ملغم /كغم/اليوم من نبات الحنظل أ ضافة الى مجموعة سيطرة. التجربة الثالثة أربعة ذكور فئران ناضجة جمعت للتزاوج مع ثمان فئران جميعهم معاملين بنبات الحنظل بتركيز 4.8 ملغم/كغم/اليوم ، وسجلت عدد الولادات الحية . جميع الفئران في التجارب الثلاث قد عوملت بنبات الحنظل عبر الفم و لمدة ثمان اسابيع. أظهرت نتائج هذه الدراسة بوجود انخفاض معنوي في معدل تركيز النطف في الفئران المعالجة بتركيز 7.2 و9.6ملغم/كغم/اليوم مقارنة بمجموعة السيطرة و الفئران المعالجة بتركيز 2.4 و4.8 ملغم/ كغم/اليوم. لوحظ وجود زيادة معنوية في نسبت حركة النطف والحركة التقدمية للفئران المعالجة بنبات الحنظل بتركيز 4.8ملغم/ كغم/اليوم بما يقابلها في التراكيز الأخرى لفئران الذكور في التجربة الأولى، وقد وجد أنخفاض معنوي في نسبة الشكلياء غير السوية في الفئران المعاملة بتركيز 4.8 ملغم/كغم/اليوم من الحنظل مقارنة بباقي التراكيز . أكدت الدراسة بوجود زيادة معنوية في عدد الولادات الحية في الفئران الذكور والاناث المعالجة بالحنظل (9.0 ± 0.4) مقارنة بالمجموعة غير المعالجة به(6±0.3). أظهرت نتائج الدراسة بان نبات الحنظل في تراكيز محددة يمكن ان يعمل على تنشيط فعالية وظائف النطف الرئيسية ويساند قابلية الخصوبة في الاناث.