TOXICOLOGICAL AND SOME REPRODUCTIVE ASPECTS OF THE EFFECTS OF ALCOHOLIC EXTRACT OF COCONUT ON MALE ALBINO MICE

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ABSTRACT

In this study, two experiments had been done: Experiment (1), was designed to determine the acute toxicity of alcoholic extract of coconut (*Cocos nucifera*) fruit to the laboratory mice. Eight groups (6 mice each) of male albino mice were used in this experiment. The first group was drenched (1ml) physiological saline (as control group), while the other seven groups were given orally ascending doses (0.25, 0.5, 1, 1.5, 2, 2.5, 3 g/kg B.W.) respectively. After (24hr), all groups of mice were inspected for the presence of dead mice. The results of this experiment showed no mortality in all groups of mice and the extract is not toxic.

Experiment (2), this study was designed to investigate the effect of alcoholic extract of coconut fruit on the efficacy of reproductive system of male mice. For this purpose, (24) mature male mice were allocated equally and randomly into three groups. Group (1) was given physiological saline and was considered as control group. Group (2) and group (3) were given 125mg/kgB.W./day and 200 mg/kg B.W. /day of alcoholic extract of coconut fruit respectively. All mice in the three groups received orally the appropriate treatment for 15 days. Post treatment, two criteria were chosen as indicators for the efficacy of the extract. The first one was relative testicular weight to body weight. The other one was the concentration of sperm in caudal part of epidydemis. The results of this experiment revealed that there was a significant ($P<0.01$) increase in relative testicular weight/body weight in group that was treated with 125mg/kg/day compared with control group. However, the second group which was treated with 200 mg/kg B.W showed significant decrease in testis weight /body weight as compared to control group and first group.

From the present study, one can conclude that the alcoholic extract of *Cocos nucifera* fruit does not toxic to the laboratory mice and the low dose (125mg/kg B.W.) causes enhancement in male fertility of mice, while higher dose (200mg/kg B.W.) causes reduction

in sperm concentration and relative testicular weight/body weight compared with the first dose.

INTRODUCTION

Actually many aromas used by ancient nations for medicinal purpose inducing problems of the reproductive system, which survive today much information on the early treatment of many ailments .

Coconut is one of the ten most useful trees in the world, providing food for million of people especially in the tropics (4). Moreover there is many different uses for coconut, they include: coconut sap (toddy) as a source of sugar, vinegar or alcohol coconut water as a delicious, non-alcoholic beverage and a substitute for blood plasma in emergency surgical operation (8). The dry copra is composed of protein, oil, carbohydrates and minerals. Carbohydrates include sucrose, raffinose, glucose, peptose, fructose, glucose, dextrin and starch (3). Oil extraction from copra contains moisture, oil, protein, carbohydrate, fibers and ash (4). On the other hand, the coconut oil is important one of many vegetable oil and it contains 0.2-0.5% cuproic, 5.4-9.5% caprylic, 4.5-9.7% capric, 44.1-51.3% lauric, 13.1-18.5% myristic, 7.5-10.5% palmitic, 1.0-3.2% stearic, 0-1.5% arachidic, 5.0-8.2% oleic and 1.0-2.6% lindeic acids. Oil extraction from copra contains moisture, 6.0-26.7% oil, 14.3-19.8% protein, 32.8-45.3% carbohydrate, fibers and ash (4). In folic medicine *Cocos nucifera* is used for many diseases such as tumor, anthelimintic, antidotal, bactericidal and aphrodisiac (6). Since the literature survey revealed little scientific work on this plant on reproductive system. Consequently, it is thought to be interesting the effect of alcoholic extract of coconut fruit on the fertility of male mice.

MATERIAL AND METHODS

Collection of plant material: Fruits of *Cocos nucifera* were purchased from local markets in Basrah Province/ Iraq. Voucher specimens of plants were identified and authenticated at College of Science/University of Basrah.

Preparation of Alcoholic extract of kernel of *C. nucifera:* The kernel part of coconut was grounded mechanically using a blender for (4 min), and then dried at room temperature for 2 days under the shade. Twenty five grams of the dried crushed material were put in the thumble of Soxhlet apparatus, 250 ml of ethanol (70%) were added and extracted for 24 hrs at 70c˚, the extract was dried using Rotary evaporator at 50c˚. The final dryness was completed by leaving the extract at room temperature. The resultant was yellowish oily material which was kept in tightly closed dark container.

Experimental animals: The laboratory albino mice (Bulb C) at (8-9) weeks of age and (23-25) gm body weight were brought from the Department of Biology, College of Education / University of Basrah.

These mice were managed in the Animal House of the College of Veterinary Medicine/University of Basrah. Mice were housed in a standard plastic cage measuring (30x13x15 Cm.) which made from propylene with stainless steel lids (North Kent plastic, Kent, U.K.). Saw dust substrate was changed weekly. Food and water were supplied *ad libitum*. The food was prepared to the laboratory mice composed from wheat flour (15%), wheat barn (25%), milk powder (2%), vitamins and mineral (1gm/Kg) of B.W. and tap water were given *ad libitium*.

Experiment (1): Determination of LD50% of alcohol extract of *C. nucifera:*

Forty two male albino mice (Bulb C) were divided equally and randomally into seven groups each group contains (6 mice) was placed in a standard cage (30x15x13 cm). The first group (control group) received orally (1ml) normal saline, while other groups were given orally ascending doses from alcohol extract of *C. nucifera* (0.25, 0.5, 1, 1.5, 2, 2.5, 3 gm /kg B.W). These groups were given orally by using a plastic disposable syringe with a blunt needle cut to a length of (5mm) and fitted with a plastic tube. Mice were left in their cages and under observation for two hours for the presence any signs of toxicity and after 24 hours for mortality rate.

Experiment (2): The right and left testis with the epididymides of each animal were removed after anaesthetizing the animal by using open ether. The testis of each animal was immediately weighed by using sensitive electronic balance after removing the fat and tissues surrounding it. The left and right epididymis was used for sperm count. The caudal epididymis was put in a watch glass containing normal saline, and then minced into small tiny pieces with microsurgical scissors until it became homogenized solution that contains the spermatozoal suspension. The concentration of spermatozoa was calculated by using haemocytometer. 0.8 ml of 10% formalin solution was added and 0.1 ml of Eosin stain (0.5%) to the spermtozoal suspension then 50µl added of the heamocytometer slide by using small pipette. The concentration of spermatozoa was calculated from the mean number of spermatozoa in twenty five small squares in each medium square of slide under magnification x40 (10). This procedure was repeated twice. Total sperm conc. =sperm conc. x solution volumes (1ml) ×10.000.

Statistical analysis: Results are expressed as mean ±standard deviation (SD). Data were statistically evaluated using SPSS computer package .Means were compared to one way analysis of variance (ANOVA) .

RESULTS

Acute toxicity (LD_{50}) of alcoholic extract of *Cocos nucifera* fruit: The doses of alcoholic extract and mortality percentage in albino mice are presented in Table (1). The results in this table showed no mortality rate from ascending doses until 3 gm/kg B.W.

Effect of oral administration of *C. nucifera* fruit extract for 15 days on testicular weight: As shown in table (2), the average concentration of sperms in the epididymis then treated with (125 mg/kg B.W.) of Coconut fruit extract was increased significant in ($P<0.01$) after 15days compared with the control group. However, the second group which was treated with (200 mg/kg B.W) showed significant decrease in testis weight /body weight as compared to the group which treated with 125 mg/kg B.W.

The results of the effect of alcoholic extract of *C. nucifera* on testis weight percentage are illustrated in Table (2). The treated group with (125 mg/kg B.W.) of Coconut fruit extract showed highly significant increase ($P<0.01$) in testis weight / body weight in treated group when compared with control group. However, the second group which was treated with (200 mg/kg B.W) showed significant decrease in testis weight /body weight as compared to the group which received 125 mg/kg B.W.

Table (1): Number of dead mice after (24 hrs) post oral administration of different doses of alcoholic extract of *Cocos nucifera* on albino mice.

Groups	Dose gm/Kg B. W.	No. of mice used	No. of mice dead	Mortality
1	0.9%NaCl	6	0	0%
2	0.25	6	0	0%
3	0.5	6	0	0%
4	1.0	6	0	0%
5	1.5	6	0	0%
6	2.00	6	0	0%
7	2.5	6	0	0%
8	3.00	6	0	0%

Table (2): Effect of alcoholic extract of *Cocos nucifera* (125 mg/kg B. W.) on sperm concentration, Testicular weight % (g/100g B.W.) **after oral administration for 38 days in male mice.**

Parameters	Control	Treatment (1) (125 mg/kg)	Treatment (2) (200 mg/kg)
Sperm concentration ($\times 10^6$)	0.947±0.804b	1.698±0.532a	0.732±0.0.344c
Testicular weight % (g/100g B.W.)	0.147±0.007a	0.262±0.005a	0.216±0.003b

Values are expressed as mean±SD, (n=10/groups).

Means bearing different letters differ significantly (P<0.01).

DISCUSSION

Determination of (LD50) of alcoholic extract of *Cocos nucifera* fruit:

The results of this study demonstrated that there was no mortality appeared in the doses used after 24 hr. From these results it can be suggested that this plant is not toxic.

(2) and(7) noticed that the oil extract of coconut is not being poisonous and does not have any side effects.

The effect of of alcoholic extract of *Cocos nucifera* fruit on male reproductive system: Two parameters have been studied; testis weight and sperm concentration. The results of this study indicate that oral and daily administration of 125 mg /kg B.W for 15 days of coconut fruit extract can increase testis weight while drenching of 200 mg /kg B.W showed reduction in the testis weight. In the case of the first dose it caused an increase in weight presumably due to the stimulation of the receptors in the testis which hold receptors for growth hormone (9). But the other dose (200 mg/kg B.W) may cause inhibition of these receptors. Similar findings were found by (1) when he used 200 mg/kg coconut extract and found there was decreasing in the weight of testis in mice.

The second parameter measured was sperm concentration which is considered as an important factor for the identification the normal function of the testis (11).

Long term administration (for 15 days) of 125mg/kg B.W of coconut extract caused an increase in the sperm concentration. From these two parameters which have been studied, the

administration of 125mg/kg B.W of coconut extract showed enhancement effects in the fertility of male mice.

التأثير السمي والتناسلي للمستخلص الكحولي لثمرة جوز الهند على ذكور الفئران البيضاء

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الخلاصة

صممت هذه الدراسة لمعرفة تأثير المستخلص الكحولي (70%) لثمرة جوز الهند في الكفاءة التناسلية لذكور الفئران وفضلاً عن دراسة التأثير السمي لثمرة جوز الهند. وعلية شملت الدراسة على تجربتين.

التجربة (1) تحديد السمية الحادة (LD50) للمستخلص الكحولي: استعملت في هذه التجربة ثمان مجاميع (كل مجموعة تحتوي ستة فئران ذكور). جرعت المجموعة الأولى عن طريق الفم (1مل) من المحلول الملحي الفسيولوجي ، إما المجاميع السبعة الباقية جرعت فموياً ايضاً جرعاً متصاعدة من المستخلص الكحولي لثمرة جوز الهند (0,25 و 0,5 و 1,0 و 1,5 و 2,0 و2,5 و 3,0 غم/كغم من وزن الجسم) على التوالي. ثم فحصت الفئران بعد أربع و عشرين ساعة بحثا عن وجود وفيات. أظهرت نتائج هذه التجربة عدم وجود فئران نافقة في كل المجاميع التي استخدمت في التجربة. ويمكن اعتبار هذا المستخلص امن وغير سام.

التجربة (2)، تحديد الجرعة المؤثرة للمستخلص الكحولي في الجهاز التناسلي الذكري للفئران: استهدفت هذه الدراسة لمعرفة الجرعة المؤثرة. لهذا الغرض قسمت (24 فأرا) عشوائيا وبالتساوي إلى ثلاث مجاميع، جرعت المجموعة الأولى المحلول الملحي الفسيولوجي واعتبرت كمجموعة سيطرة أما المجموعة الثانية والثالثة فقد جرعت 125ملغم/كغم و200ملغم/كغم من وزن الجسم على التوالي يوميا ولمدة 15 يوما. ولغرض معرفة تأثير المستخلص على الجهاز التناسلي الذكري، اختيرت فئتان من الفحوصات كمؤشر لذلك. الأولى كانت نسبة وزن الخصية /وزن الجسم أما الفحص الثاني كان تركيز النطف في الجزء ألذيلي من البربخ.

أشارت النتائج التجربة ان هناك زيادة معنوية عالية ($P<0.01$) في نسبة وزن الخصية /وزن الجسم ومعدل تركيز النطف البربخية في المجموعة التي عولجت بـ 125ملم/كغم مقارنتا بمجموعة السيطرة، بينما المجموعة التي عولجت بـ 200ملم/كغم أظهرت انخفاضاً معنوياً عالياً ($P<0.01$) في معدل تركيز الحيامن نسبة وزن الخصية /وزن الجسم ومعدل تركيز النطف البربخية مقارنة بمجموعة السيطرة والمجموعة الأولى.

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