Effect of *Melia azedarach* on The Reproductive Efficiency in Female Rabbits

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Abstract

Various types of contraceptives were developed. These contraceptives are prepared from natural as well as synthetic sources. The study was conducted on 15 mature adult females, and males of rabbits.10 from each female and male rabbits were treated with *Melia azedarach* in powder form at a dose rate of 6 g / kg b.wt. Orally mixed with feed daily for 53days, the remaining 5 from each of female and male rabbits remain non treated. In 28th day post exposure to Melia; the animals were divided into 3 groups. Group I represented females non treated with plant crossed with treated males. Group II represented treated females crossed with non treated males. Group III represented non treated females crossed with non treated males. The mixing continued for 10 days. All females were examined for pregnancy by sonar weekly. At 56th day post treatment with plant 3 females from each group were killed, while others 2 female rabbits were left till birth. Size, weight and characters of naturally birthed fetuses and those found during explore the uterine horns of killed rabbits were fixed. Other dependent parameters in this study were hematological and some of constituents of serum. The results of the study revealed a non significant decrease in body weight, body temperature, heart rates, respiratory rate, clotting time and bleeding time. Erythrocytes counts, PCV, MCV values were not significantly increased in treated group, while in non treated group these were not significantly decreased. Hb, MCH, MCHC were not significantly changed in both groups. Total leucocytes count in treated group were decreased, whilst increased in non treated group. Heterophils% were decreased in treated group. Lymphocytes percentage in treated group were increased, on other hand monocytes, eosinophil and basophils percentages did not show any significant

changes. The results revealed that infertility index was 0, 20 and 100% in group I, II and III respectively. Significant decrease in numbers, body weight, body length and width of skull of fetuses from females treated with extract of Melia, and those crossed with treated male in comparison with those of non treated females and males. The results revealed that treatment with Melia extract for 53 days did not affect the length of pregnancy in female treated with Melia extract. The results revealed that levels of ALT, AST and AP were higher in treated females in comparison with those of non treated rabbits. Values of FSH, LH, Prolactin and testosterone were less than normal levels,

Key words: Reproductive efficiency, Female rabbits, *Melia azedarach*

تأثير نبات السبحبح *Melia azedarach* في الكفاءة التناسلية لإناث الأرانب

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الخلاصة

تم تطوير أنواع مختلفة من موانع الحمل ، والتي تختلف في طريقة تأثيرها. تحضـــر هذه الموانع من مصـــادر طبيعية وصناعية . أنجزت الدراسة على 15 أرنبا ناضجا من كل من الذكور والإناث . عوملت 10 أرانب من كل جنس بمسحوق نبات السبحبح (*Melia azedarach*) ممزوج مع العلف ، وبجرعة يومية بمعدل 6 ملغم \ كغم من وزن الجسم ، ولمدة 53 يوم . وترك 5 من ما تبقي من كل من الذكور والإناث بدون معاملة. في اليوم 28 بعد المعاملة قســمت الحيوانات إلى ثلاثة مجاميع . المجموعة الأولى مثلت الإناث غير المعاملة والتي زوجت مع ذكور معاملة بمسحوق النبات، أما المجموعة الثانية فمثلت الإناث المعاملة بمسحوق النبات والتي زوجت مع ذكور غير معاملة ، في حين في المجموعة الثالثة تم تزاوج ذكور وإناث غير معاملة بمسحوق النبات. استمر التزاوج لمدة 10 أيام ، بعدها أخضعت جميع الإناث للفحص بالسونار أسبوعيا بحثا عن حصول الحمل . في اليوم 56 من بدء التعامل بمسحوق النبات تم ذبح 3 إناث من كل مجموعة بحثا عن حصول الحمل من عدمه ، وعد الأجنة ووزنها وتثبيت خصائصها من طول الجسم وعرض الجمجمة في حالة حصول الحمل . أما ما تبقى من الإناث 2 من كل مجموعة فترك لحين إكمال الحمل والولادة الطبيعية . من المعايير الأخرى التي اعتمدت في الدراسة الفحوصات الدموية والكيميوحيوية في مصل الدم. أظهرت نتائج الدراسة هبوط غير معنوي بوزن الجسم ، ودرجة حرارة الجسم ، وضربات القلب ، وسرعة التنفس ، وزمن التخثر ، وزمن النزف. في حين حصلت زيادة غير معنوية في قيم العدد الكلي لكريات الدم الحمر، وحجم الخلايا المرصوصة ، ومعدل حجم الكريه في المجاميع المعاملة ، لكنها هبطت هبوطا غير معنويا في المجاميع غير المعاملة. بينما لم يحصـــل تغير معنوي في قيم خضـــاب الدم ، ومعدل

خضـــاب الكريه، ومعدل تركيز خضـــاب الكريه في كلا من المجاميع المعاملة وغير المعاملة . هبط العدد الكلي لخلايا الدم البيض في الحيوانات المعاملة بمسحوق النبات ، لكنه ارتفع في المجاميع غير المعاملة . هبطت نسبة العدلات في المجاميع المعاملة ، اما نسبة اللمفية فارتفعت في المعاملة بمسحوق النبات . الخلايا احادية النواة ، والحمضـات ، والقعدات لم تتغير نسبها في كلا من المعاملة وغير المعاملة بمسحوق النبات . أظهرت نتائج الدراسة إن مؤشر الخصوبة بلغ 0 ، 20 ، و 100% في إناث المجموعة الأولى، والثانية ، والثالثة على التوالي. حصـــل انخفاض معنوي في اعداد، واوزان الجســـم ، وطول الجســـم ، وعرض الجمجمة للأجنة من اناث معاملة بمســـحوق النبات، وتلك التي زوجت مع ذكور معاملة مقارنة بالإناث غير معاملة والتي زوجت من ذكور غير معاملة. اظهرت النتائج ان المعاملة بمسحوق النبات لمدة 53 يوما لم يؤثر على مدة الحمل في الاناث المعاملة . اظهرت النتائج ايضـــا ان مســـتويات الخمائر الناقل الامينيز الالنين والاســـبرتيت والفوسفات القاعدي كانت اعلى في الحيوانات المعاملة مقارنة بتلك غير المعاملة. قيم هرمونات محفز الجريب ، واللوتيني ، والبرولاكتين ، والتستيرون كانت اقل من الطبيعي .

الكلمات المفتاحية: الكفاءة التناسلية ، اناث الأرانب ، السبحبح (*ميليا ازيدراج*)

Introduction

The contraceptives were worked by prevent the fusion of sperm into ovum, change female hormonal levels and spermicidal activity (1). Rapid rise in population has caused serious problem in economic growth and human development. Family planning has been promoted through several methods of contraception, but due to serious adverse effects, such as hormonal imbalance, hypertension, and increased risk of cancer and weight gain, the search for new anti-fertility molecule with minimum side effects continues. Hydro alcoholic extract of *Melia azedarach* linn roots were evaluated for anti-implantation, estrogenic, ant estrogenic and progestational, anti-progestational activity. It was found that the extract has very significant ant implantation and anti-progestational activity and devoid of estrogenic, anti- estrogenic activity. (2). *Melia azedarch* L. belong to family Meliaceae, subfamily Meloideae (3). Herbs are used for thousands of centuries by many cultures for their medicinal values. Herbal treatment is very popular because it is easily available, cheap and less toxic (4).

Several workers reported the anti-fertility activity of different parts of this plant. Many researchers reported that there was significant decrease in the number of normal follicle in ovaries of rat, if polar and nonpolar fractions, of *Melia azedarach* linn seed extract was

administered at 24 mg / kg for 18 days (5). Ethanolic extract of *Melia azedarach* intercepted pregnancy in 60% and 75% adult female rats (6). The seed oil of *Azadirachta indica* A. Juss (neem) is used in traditional medicine for its anti- diabetic, spermicidal, anti-fertility, antibacterial, and wound healing properties (5). The effect of oral administration of *Melia azedarach* Linn (dharek) seed extract on fertility index, uterine weight and various histological and biochemical parameters of uterus were studied in the adult cyclic Wistar rats. Average number of embryos and implantation losses in the pregnant animals treated with dharek seed extract was also studied (2). Antifertility activity of *Azadirachta indica* (neem) and *Melia azedarach* (dharek) on the ovaries has been reported earlier (5, 7).

Material and Methods

The study was conducted on 15 mature female, and 15 mature male rabbits of 1-2 kg b.wt, 1-2 years old. after acclimatization for 2 weeks under room temperature of 25± 1^{o}C, and 12 h light, 12 h dark conditions. 10 from each of males and females were treated with *Melia azedarach* in powder form at a dose rate of 6 g / kg b.wt. orally mixed with feed daily for 53days, 5 from remaining females and males remain without treatment as non treated. In 27th day post treatment with Melia; the animals were divided into 3 groups: male with female 1:1. Group I represented treated females crossed with non treated males. Group II represented non treated females crossed with treated males. Group III represented non treated females crossed with non treated males. The crossing continued for 10 days. Females of all three groups were examined for pregnancy by sonar weekly. At 56th day post exposure to plant (28th day post crossing with male). 3 females from each group were killed, while others 2 females from each group were left to complete pregnancy time till normal birth.

Examine the Pregnancy Period and Number of Fetuses

Genital system of killed rabbits from each of the three groups were examined, as the abdomen was opened, explore the uterine horns, which opened to measure the fetuses and their numbers if there were pregnancy, in addition to calculate resorption number if present.

Others dependent parameters in the study include hematological examination according to (8), in addition to some constituents in serum (kits methods)

Statistical Analysis

The data were analyzed by one way analysis of variance. The means were separated following Duncan test (9), at the level of $P < 0.05$

Results

Results of this study revealed not significant decrease in body weight of treated group at 44th day in comparison with previous days, but in both treated and non treated groups there were not significantly increase in comparison with previous days. Body temperature in treated and non treated groups was decreased in the 39th day. Heart rates decreased in treated group in 56th day in comparison with previous days and with values on non treated group. Rrespiratory rate in treated group was not decreased significantly in the 39th ,44th and 56th day in comparison with previous days (Table-1-).

Table -1- Clinical Parameters of Rabbits in Experiment

Parameter	Group	Day			
		0	39	44	56
Body Weight Kg	I	1.366 ±0.108	1.316 ±0.109	1.298±0.140	1.445 ±0.005
	II	1.370±0.106	1.355±0.085	1.326±0.103	1.450±0.115
Body Temperature ºC	I	38.73±0.36	37.96 ±0.42	38.7±0.40	38.27±0.42
	II	38.74 ±0.44	37.99 ±0.23	38.42±0.18	38.02±0.29
Heart Rate/min	I	176±11.66	177.5±6.88	183±10.75	161.33±1.33
	II	180±9.17	175.1 ±11.28	188.8±7.84	180.8±11.20
Respiratory. Rate/min	I	172±18.0	130±5.77	137±15.78	136.34±16.71
	II	173.6±13.30	168.2±8.23	168±10.66	173.6±13.00

Values are M ± SEM: I treated group; II. Non treated group.

Clotting time in treated group significantly decreased in the 23rd, 44th and 56th in comparison with previous values and with values of non treated group. Bleeding time in treated group not significantly decreased in the 44th day in comparison with previous days (Table-2-).

Table -2- Clotting and Bleeding time of Rabbits in Experiment

Parameter	Group	Day			
		0	23	44	56
Clotting Time/ sec.	I	45±1.89a	20±1.94b	28.75±2.57b	15.67±1.41b
	II	40±1.17a	42 ±1.17a	45±1.04a	50±1.32a
Bleeding Time/ sec	I	37 ±0.39	36.25±1.34	31.67±1.51	36.67 ±2.99
	II	35 ±0.79	32 ±1.79	33±2.57	35±1.31

Values are M ± SEM: I treated group; II. Non treated group: a, b significant difference at a level of P < 0.05 in comparison with previous day

Erythrocytes counts in treated group was significantly decreased in the 23rd, 44th, 56th day in comparison with previous days, the lowest counts was in the 44th day, however in non treated group no changes was observed . Hemoglobin concentration was not changed significantly. PCV in treated group significantly decreased in the 56th day, in comparison with previous day. MCV values in treated group was not significantly increased in the 23rd and 56th day, but not significantly decreased in the 44th day in comparison with previous day. There was not significant decreased In non treated group in day the 56th day. whilst not significant changes in MCH, MCHC in both groups (Table-3).

Table -3- Total Erythrocytes Count, Hb Concentration, PCV% and Erythrocyte Indices of Rabbits in Experiment.

Parameter	Group	Day			
		0	23	44	56
RBCX10^6/ mm^3	I	5.11±0.31a	4.3±0.26b	3.85±0.06b	4.2±0.29b
	II	5.12±0.44a	4.94±0.25a	4.79±0.43a	5.23±0.64a
Hb g/dl	I	10.38±0.44	10.13±0.70	9.3±0.51	9.68±0.39
	II	9.36±0.45	10.32±0.32	9.18±0.81	9.77±0.71
PCV%	I	33.2±1.36	32±2.27	33.67±0.88a	30.5±1.04b
	II	33.2±1.36	32.6±1.29	31.6±2.84	31.5±2.02
MCVft	I	66.37±6.15a	74.32±1.87a	62.70±5.38a	73.65±4.37
	II	69.73±6.68a	72.84±5.27a	77.66±2.02a	70.02±5.41
MCHpg	I	20.76±1.95	23.53±0.56	19.43±1.74	20.20± 1.10
	II	21.69±2.03	23.02±1.44	22.91±0.68	21.47±1.56
MCHCg/dl	I	31.25±0.06a	31.65±0.11a	30.98±0.15a	31.02± 0.6a
	II	30.97±0.11	31.71±0.5	31.11±0.14	31.04±0.21

Values are M ± SEM: I treated group; II. Non treated group; a, b significant difference at a level of P < 0.05 in comparison with previous day.

Total leucocytes count was decreased in treated group in the 23rd ,44th and 56th day in comparison with previous day, whereas in non treated group increased in the 23rd and 56th day. Heterophils% decreased in treated group at 23rd day. Lymphocytes percentage in treated group was increased at 23rd day. Monocytes, eosinophil and basophils percentages did not show any significant changes (Table-4).

Table -4- Total Leucocytes Counts and Differential Leucocytes Count of Rabbits in Experiment.

Parameters	Group	Day			
		0	23	44	56
WBC X10^3/ cmm	I	5.106±0.48	4.832± 0.422	4.118±0.368	4.22± 0.367
	II	4.668±0.434	5.044±0.689	4.786±0.115	5.833±0.555
Heterophils%	I	43.6±8.27a	32.75±3.64a	40.67±0.33a	41.85± 0.27a
	II	41.4±4.46a	40.2±5.54a	40.4±4.15a	41.57±5.56a
Lymphocytes. %	I	50.4±8.29a	61.75±4.33a	52.67±0.88a	53.86 ±0.76a
	II	46.2±4.05a	52.2±5.85a	48±3.46a	47±5.94a
Eosinophils. %	I	2.8±o.37a	2.75±0.48a	4.33±0.58b	2.25±0.25a
	II	2.6±0.40a	2.6±0.51a	2.37±1.31a	3±0.71a
Basophils. %	I	1±0.32	0.75±0.48	0.66±0.11	0.75 ± 0.25
	II	1.5±0	1.8±0.37	1.27±0.35	0.72±0.27
Monocytes. %	I	2.2± 0.2	2±0	3±0.58	2 ±0.21
	II	2.5± 0.55	2.8±0.80	2.4±0.42	3.29±0.48

Values are M ± SEM: I treated group; II. Non treated group; a, b significant difference at a level of P < 0.05 in comparison with previous day

The results revealed that there was no pregnancy and infertility index was 0% in group I (non treated females crossed with treated males). While in group II (treated females crossed with non treated male) there was one rabbit pregnant from 5 (infertility index 20%. Meanwhile in group III (both male and female non treated) they fertility index was 100% (Table-5-).

Table -5- Numbers of Pregnant, Non-pregnant Females, Total Numbers, and Infertility Index of Rabbits in Experiment.

Groups	Pregnant	Non pregnant	Total	Infertility index
I	0	5	5	0%
II	1	4	5	20%
III	5	0	5	100%

I. Females non treated crossed with treated male

II. Female treated crossed with non treated male

III. Female non treated crossed with non treated male

The results revealed significant decrease in fetus numbers in female treated with Melia extract, and those crossed with treated male in comparison with those of non treated female and male. The results revealed decrease in body weight, length of the body, and width of skull of fetuses found in uterine at time of killing of females, and treated females, giving normal birth in comparison with those from non treated female. The results revealed that treatment with Melia extract for 53 days did not affect the length of pregnancy in female treated with Melia extract as the periods remain 30-32 days. (Table-6-).

Table -6- Showing Weight, Length, and Width of Skull of Fetuses obtained from Female Rabbits in treated and Non treated Groups

Parameters	Non treated	treated
Body Weight of Fetuses \gm	43.33±2.4	28 ± 2.5
Body Length of Fetuses\ mm	90.67±3.44	83.6 ± 1.89
Width of Fetuses Skull\ mm	24.5±0.22	14.67 ± 9.42

The results revealed that levels of ALT, AST and AP were higher in treated females in comparison with those of non treated females but they are within normal ranges except AP was higher than normal level. Values of FSH, LH, Prolactin and testosterone less than normal levels, but there were no differences between those of treated and non treated females (Tbale-7-).

Table -7- Levels of ALT, AST, AP, FSH, LH, Prolactin and Testosterone of Female Rabbits in treated and Non treated Groups.

Groups\ Parameter	Female		Normal values
	Treated	Non treated	
ALT u/l	32.3	9.95	10-35
AST u/l	16.7	5.22	0-40
AP u/l	166	71.95	35-129
FSH mlU/ml	< 0.1	<0.1	6.3-24
LH mlU/ml	<0.1	<0.1	9.6-70
Prolactin ng/ml	0.047	0.047	0.7-19
Testosterone ng/ml	<0.025	<0.025	0.1

Discussion

The reduced of pregnancy volume in female crossed with male treated with *Melia azedarach* can resulted from many variants in fertility, in the top of which the reduction in sperm counts, increased dead sperm numbers, and the percent of sperm deformity, in addition to disturbance of epididymis functions under the effects of androgens (10). The reduction in fertility also increased due to disturbances of functions of accessory sexual organs which supply seminal plasma which is important for continuity of sperms life (10; 11). Increase in embryonic resorption and reduced weight of embryos can attributed to sperm deformity, as many of these deformity can lead to inhibition of embryonic development, or may be results from deformity of endometrium functions before arriving of embryos (12). and this proved by the histological changes in uterus which showed hyperplasia of uterine glands, and degeneration with thickening of myometrium. The intoxication of females can lead to embryonic resorption and reduced their weights and their death (13). Reduction in fertility was observed due to leucocytes infiltration in uterus resulting in inhibition of implantation and blockage of pregnancy (14, 15). Secondly, anti- implantation activity has been reported due to histopathological changes produced by inhibition of estrogen which induced changes in the uterus (16). Thirdly, the cause of implantation losses was observed due to steroidogenic depression evidenced by reduced plasma progesterone: estrogen ratio (17). Fertility index in adult cyclic rats after 18 days of treatment by dharek seed extract was reduced with the increase in dose as compared with the control group (4, 18). This supports the findings of (2) who observed reduction in fertility after

vaginal administration before mating and unilateral administration of neem oil in the uterus of female Wistar rats.

Serum levels of luteinizing hormone were lower significantly in group treated with methanol leaf extract of *Azadirachta indicia* orally at 200 and 400 mg /kg, while there were significant higher progesterone levels, follicle stimulating hormone levels were however not different from the control. the histomorphic studies revealed no obvious pathological changes in the ovaries and uteri of the treated groups. (200 mg / kg, and 400 mg / kg of methanol extract of the leaf of A. index does not have any obvious effect on the histomorphologies of the ovary and uterus, but showed significant changes in the serum levels of LH and PH of female Wistar rat, implying that the effect of the extract may have been at a level other than these organs of the study (2). (14, 19) reported normal uterine and ovarian morphologies, and functions with the seed oil extract of *A. indica*. (20) reported normal histoarchitecture of the uterus of rats treated with neem oil extract (21) reported reduction in serum LH levels after treatment with extract of *A. indica*, while (20) reported that neem oil did not possess any estrogenic , anti – estrogenic or progestsational activity , while appearing not to have interfered with the actions of progesterone (5, 14, 22) reported folliculogenesis inhibition, prolonged diestrus and partial blockage of ovulation, as well as, enhanced antigen – presenting ability of the uterus with seed and oil extracts of *A. indica.*

Significant damage to the luminal epithelium of the uterus and to the uterine glands, with decreased glycogen and total protein contents in the ovary and uterus, has also been reported on administration of oil of neem to cyclic and ovariectomized rats (23), while (24) reported alterations in morphologies and functions of the uterus in rats treated with the seed extract of neem.Number of retrieved unimplanted embryos has been found to have the attached leucocytes to the zona pellusida layer it is believed that this secretion of leucocytes might be responsible for under development of early embryo or by initiating a cellular immune response in the uterus leading to blocking of implantation (14, 15).

The anti-estrogenic quality of neem oil also explains its anti- implantation effect. But the post implantation effect, which caused implanted fetuses to be either resorbed or expelled, also may be due to direct toxicity, fall in progesterone level or interference with the uterine utilization of progesterone (25).

The treated males with *Melia azedarach* in this study lead to increase the percent of dead sperms in epididymis tail, and this may be due to presence of spermicidal material. (26).

Conclusion

Melia azedarach lead to increase the percent of dead sperm in epididymis tail, so work as antifertility and anti-implantation

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