Antifertility Activity of *Melia azedarach* in Male Rabbits

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Abstract

This study was conducted on 10 albino adult male rabbits, of 1-2 kg b. wt., 1-2 years old. The animals were divided in two groups, treated group was treated with *Melia azedarach* in powdered form at a dose rate of 6 g / kg b.wt. orally mixed with feed daily for 53 days. While those of control group were left without treatment. All animals were killed at 56 days of experiment Blood samples were collected to obtain the serum for biochemical examination. In addition to weight the testes, head, body, tail of epididymis, seminal vesicle and prostate. Total number of sperms in epididymis head was accounted. Calculation of life sperm and deformed sperms. The dependent parameters were included clinical and hematological examination. The results of the study revealed that in treated group clotting time was significantly decreased. While bleeding time was non significantly increased in 23rd day. Erythrocytes counts, Hb concentration, PCV%; MCH were non significantly increased. MCV increased in day 23rd but decreased in 56th day. Meanwhile MCHC non significantly decreased. Total leucocytes count was not changed in both treated and control groups. lymphocyte percent in treated group was non significantly increased, while Heterophils and eosinophils percent were non significantly decreased. Basophils and Monocytes percentages were significantly decreased. The results revealed that the weight of sexual organs and the accessory glands, in addition to total sperm counts of treated males were less than those of control males. While the dead sperm and deformed sperm were more in treated than in control males. The results revealed that ALT, AST and AP values were within normal range but of lower level in treated males in comparison with those of control males. Histopathological examinations revealed that the main changes in sexual organs of treated male were included, appearance of spermatid giant cells with few sperms in the lumen of seminiferous tubules, round spermatid cells in the lumen of seminiferous tubules with vacuolation of their epithelial cells. cellular debris in the lumen of epididymis with vacuolation of the epithelial lining. While in control males the main histological changes in sexual organs were included sperms filled the lumen of epididymis, and normal structure. In the testis shows complete spermatogenesis with sperms in the seminiferous tubules. In seminiferous tubules shows normal structure and their lumen contain sperms. From results we can conclude that *Melia azedarach* has an anticonception material that effect the total number of sperm with inducing a deformity in sperm parts, with less side effects on health of treated males.

Key words: Antifertility activity, Melia azedarach, male rabbits
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الفعالية المضادة للخصوبة للميليا ازيدراج في ذكور الأرانب

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الخلاصة

أجريت الدراسة على 10 ذكور من الارانب ناضجة، بوزن 1- 2 كغم، وعمر 1-2 سنة. قسمت الحيوانات إلى مجموعتين: مجموعة معالجة ومجموعة سيطرة. عرضت حيوانات مجموعة المعاجلة إلى مسحوق ثمار نبات المليليا ازيدراج وبجرع بمعل 6 غم/ كغم عن طريق الفم مع العلف يوميا لمدة 53 يوم، بينما تركت حيوانات مجموعة السيطرة بدون معالجة. ذبحت جميع الحيوانات في اليوم 56 من التجربة، وجمعت عينات من الدم للفحوصات الدمية والكيميوحيوية. تم وزن الخصى، والبربخ (الرأس، الجسم، والذيل)، والحويصلة المنوية، والمؤثة. فضلا عن عد النطف في البربخ، وعد النطف الحية وعد النطف التي أظهرت تشوهات في أجزائها. اعتمدت معايير إضافية في الدراسة شملت معايير سريريه ودمية. اظهرت نتائج الدراسة إن زمن التخثر انخفض معنويا في المجموعة المعالجة واستطال زمن النزف في اليوم 23 من التعرض. ارتفع عدد كريات الدم الحمر، وتركيز خضاب الدم، وحجم الخلايا المرصوصة، ومعدل خضاب الكريه في الحيوانات المعالجة. ارتفع معدل حجم الكريه في اليوم 23، لكنه انخفض في اليوم 56 في المجموعة المعالجة. انخفض معدل تركيز خضاب الكريه في المجموعة المعالجة. لم يلاحظ أي تغيير في عدد خلايا الدم البيض في كلا المجموعتين. نسبة الخلايا اللمفاوية ارتفع في المجموعة المعالجة، بينما انخفضت نسب العدلات والحمضات. أظهرت نسب القعدات وأحادية النواة انخفاض معنوي في المجموعة المعالجة. أظهرت النتائج إن أوزان الأعضاء التناسلية والغدد اللاحقة فضلا عن عدد النطف الكلي في الذكور المعالجة كان اقل مما في ذكور السيطرة. وكانت النطف الميتة والتي أظهرت تشوهات في أجزئها أكثر في الذكور المعالجة مما في ذكور السيطرة. أظهرت النتائج إن مستويات ناقل الامينيز الالنيني، وناقل الامينيز الاسبرتيتي، والفوسفوتيز القلوي كانت ضمن المديات الطبيعية، لكن مستوياتها كانت ادنى في الذكور المعالجة مقارنة بذكور السيطرة. اظهر الفحص النسيجي المرضي ان التغييرات الرئيسية لأعضاء التناسل في الذكور المعالجة شملت: ظهور خلايا عملاقة مع عدد قليل من النطف في تجويف الانابيب المنوية، وخلايا نطفية مدورة مع تجويف خلاياها البطانية. وفي البربخ هناك حثالة خلوية في التجويف مع تجويفات للخلايا البطانية. بينما شملت التغيرات النسيجية الرئيسية في الاعضاء التناسلية لذكور السيطرة امتلاء التجويف بالنطف، والتركيب طبيعي. في الخصى ظهرت عملية كاملة للتكوين النطف في الانابيب المنوية، والتي اظهرت تركيب طبيعي واحتواء تجويفها على النطف. ويمكن الاستنتاج ان نبات الميليا ازيدراج يحتوى على مواد فعالة لها تأثير كمانع للحمل من خلال تأثيره على عدد وشكل الحيوانات المنوية مع تأثيرات جانبية بسيطة على الحيوانات المعالجة.

مفتاح الكلمات: الفعالية المضادة للخصوبة، الميليا ازيدراج، ذكور الأرانب

Introduction

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 antifertility molecule with minimum side effects continues. *Melia azedarach L*. belong to family Meliaceae, subfamily Meloideae (1). *Melia azedarach* Linn, belong to family Meliaceae. Several workers reported the antifertility activity of different parts of this plant. In one study, ethanolic leaf extract of *Melia azedarach* were investigated for antifertility activity on male rats in oral dose of 100 mg / kg daily for 21 days. There was abolition of libido in

100% males (2). The motility of rat and mice spermatozoa was inhibited with various concentrations of petroleum ether fractions of *Melia azedarach* seed at different time intervals ranging from 20 seconds to 240 seconds as compared to control. The effect was dose dependent and complete spermatozoa immobilization was seen with 10 to 25 mg concentrations tested for 240 -209 seconds, respectively (3). It has been reported that sperm motility of rats showed a significant difference for those receiving *M. azedarach* compared to that of the controls. Daily sperm production showed a significant reduction for those on *M. azedarach* in comparison with control group. The results also demonstrated a significant reduction in fertility rate compared to the controls (4). The seed oil of *Azadirachta indica A.* Juss (neem) is used in traditional medicine for its antidiabetic, spermicidal, antifertility, antibacterial and wound healing properties (5). Various types of contraceptives were developed having different mode of action. This contraception worked by prevent the fusion of sperm into ovum, change female hormonal levels and spermicidal activity (6). Azadirachta indica showed various pharmacological activities and have demonstrated to possess good spermicidal activity also. But there is still a need to develop alternative compounds for future use as safe spermicide. The aim of the study was to demonstrate if there is an anticonception effect of Melia azedarach on the sexual activity of males, in addition of its side effects if present.

Material and Methods

The study was conducted on 10 albino adult male rabbits, of 1-2 kg b. wt, 1-2 years old. Post adaptation for 2 weeks under room temperature of 25± 1^{o}c, and 12 h light, 12 h dark conditions. The animals were divided in two groups, first group was treated with *Melia azedarach* in powdered form at a dose rate of 6 g/ kg b. wt orally mixed with feed daily for 53 days, while those of control group were left without treatment. All animals were killed at 56th days of experiment, blood samples were collected for biochemical examination. Testes, head, body and tail of epididymis, seminal vesicle and prostate were weighed. Histological sections were taken and done according to (7).

- **Calculation sperm content of epididymis head:** Method of Sakamoto and Hashimoto (8) was depended in this field. The total number of sperms in epididymis head was accounted according to (9). Calculation of life sperm ratio and the ratio of deformity in sperms according to (10). The additional parameters in the study were clinical and hematological parameters according to (11).
- **Statistical analysis:** All values are expressed as the mean ± the standard error of the mean (SEM) using t test in comparison of the means for statistical differences according to (12). The significant level of test was $P < 0.05$.

Results

Body weight; body temperature; heart rates and respiratory rate showed none significant changes. Clotting time in control group decreased in 23rd and 53th day, in treated group it decreased significantly in 23rd and 53th day, the lowest level was in 23rd day of study. Bleeding time in treated group increased in 23rd day (Table 1). Erythrocytes counts; in treated group increased in 53th day. Hb concentration, PCV%; MCH increased in 23rdday in treated group. MCV increased in day 23rd but decreased in 53th day in treated group. MCHC decreased in 23rd and 53th day in treated group. All above values showed no changes in control group (Table 2). Total leucocytes count showed no changes in both treated and control groups, lymphocyte percent in treated group increased in 23rd and 53th days, Heterophils and eosinophils percentages in treated group decreased in day 53th, Basophils and Monocytes significantly decreased in 53th day in treated groups (Table 3). The results revealed that the weight of sexual organs and the accessory glands, in addition to total sperm counts of treated males were less than those in control males. While the dead sperm and deformity in sperm parts were more in treated than in control males (Table 4).

The results revealed that ALT, AST and AP values were within normal range but of lower level in treated males in comparison with those of control males (Table 5). Histopathological examinations revealed that the main changes in sexual organs of treated males were included: the testis showed spermatid giant cells with few sperms in the lumen of somniferous tubules, in the other section round spermatid cells in the lumen of somniferous tubules with vacuolation their epithelial cells. The epididymis showed cellular debris in the lumen with vacuolation of the epithelial lining (Fig: 1-A, B, C). While in those of control males the main histological changes in sexual organs were included: The epididymis shows sperms filled their lumen, and normal structure. The testis showed complete spermatogenesis with sperms in the somniferous tubules. The seminiferous tubules showed normal structure and their lumen contain sperms (Fig: 1-D, E, F).

Table (1) Changes in clinical signs (bodyweight, body temperature, heart rates and respiratory rates); clotting and bleeding times of animals used in study

Parameters	Group	Day		
		0	23	53
B.W. kg	I	1.318±0.039	1.408±0.025	1.303±0.130
	II	1.338±0.133	1.404±0.125	1.400±0.125
B. Temp. ^{o}C	I	38.4±0.32	38.21 ±0.26	38.38±0.24
	II	38.22±7.85	39±0.49	38.9±0.25
Heart Rate/ min	I	186±8.72	198.75±7.40	195.0±5.30
	II	187.5±4.79	192±10.20	190±6.65
Resp.Rate/ min	I	188±12.00	185.25±23.97	180.78±10.15
	II	172.4±23.45	196±13.27	190±8.85
Bleeding time/sec.	I	31±4.58	40±7.64	32.5±10.89
	II	30±6.12	29±3.25	31± 5.10
Clotting Time /sec	I	45±10.0	30.1±6.75	32.5±10.90
	II	45±10.0	36.67±14.24	40±10.25

Values M ± MSE.I: treated group; II: control group

Table (2) The total erythrocytes counts, Hb concentration, PCV, in addition to erythrocytes indices of rabbits in used in study

Parameter	Group	Day		
		0	23	53
RBC x10^{6}/ Cmm	I	3.88±0.31	3.85±0.06	4.2±0.29
	II	5.126±0.623	4.950 ± 0.525	4.890 ±0.310
Hb g/dl	I	9.44±1.46	10.33±0.29	9.68±0.39
	II	11.24±0.46	10.95 ± 0.23	11.05± 0.35
PCV%	I	30.4±0.57	33.67±0.88	30.5±0.03
	II	35.8±1.39	36.01± 0.95	35.5 ± 0.85
MCV ft	I	80.06±6.69	87.35±1.76	73.65±4.37
	Ii	72.79±6.33	75.56 ± 3.55	73.15± 5.55
MCH pg	I	24.85±2.17	27.33±0.61	23.38±1.55
	II	22.84±1.99	23.56± 1.55	24.11± 1.23
MCHC g/dl	I	31.02±0.13	21.28 ±0.07	22.75 ± 0.06
	II	31.38±0.07	30.95 ± 0.05	31.02±0.12

Values M ± MSE. I: treated group; II: control group

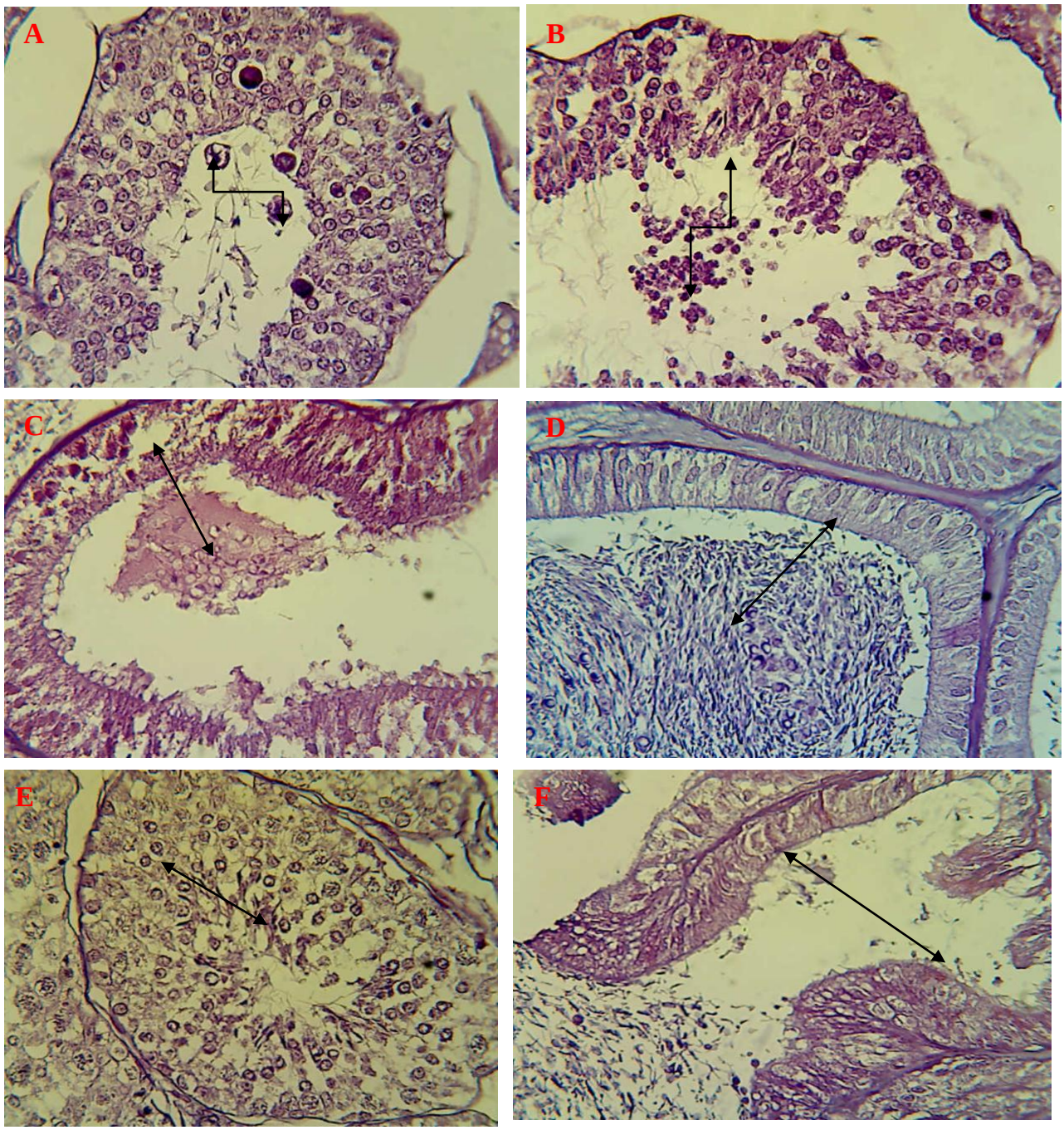

Fig: 1-A-. Histopathological section in the testis shows spermatid giant cells with few sperms in the lumen of somniferous tubules. **B**-Histopathological section in the testis shows round spermatid cells in the lumen of somniferous tubule with vacuolation their epithelial cells. **C**- Histopathological section in the epididymis shows cellular debris in the their lumen with vacuolation of the epithelial lining. **D**-Histopathological section in the epididymis shows sperms filled their lumen**. E**-Histopathological section in the testis shows complete spermatogenesis with sperms in the seminiferous tubules. **F**-Histopathological section in spermatic cord of animal shows normal structure and their lumen contain sperms**. (H&E stain 40X).**

Table (3) Total leucocytes count and differential leucocytes count in animals in use in study

Parameters	Group	Day		
		0	23	53
WBCx10^3/cmm	I	2.821±0.535	2.733 ±0.214	2.755±0.321`
	II	3.048±0.337	2.95 ± 0.45	2.755±0.320
Heterophils%	I	39.5±1.17a	39.5± 5.25a	34.67 ± 7.54a
	II	40.2±4.93	40.3± 3.5	39.5±5.25
Lymph.%	I	46.2±1.83a	54.33 ±10.17a	55.75 ± 4.28
	II	47.8±4.86	49.5± 5.45	48.5±5.25
Eosinophil.%	I	5.4±0.68a	4.33 ± 1.86a	2.25 ±0.25b
	II	2.4±0.40	2.35± 0.35	2.25±0.25
Basophil.%	I	3.2±0.20a	1.33± 0.33b	0.5± 0.41 b
	II	2.2±0.37	1.5± 0.25	0.5±0.29
Monocyte.%	I	5.6±1.44a	5 3±0.61a	2 ±0.41b
	II	2.4±0.40	2.1 ±0.15	2±0.41

Values M ± MSE. I: treated group II: control group: a,b significant in comparison with pre exposure vale with in same group. significant difference at a level of P<0.05.

Table (4) Weights of sexual organs in grams with the morphology and counts of sperms

Organ	Part	Treated	control
Testis		1.245±0.11	1.96 ±0.09
	Head	0.15±0.02	0.40 ± 0.01
Epididymis	Body	0.095±0.028	0.22 ± 0.01
	Tail	0.31±0.033	0.52 ±0.01
Prostate		0.48 ± 0.04	0.75 ±0.02
Seminal vesicle		0.22± 0.02	0.56 ±0.03
Sperm count		150992 ± 100	900000 ± 105
Morphology	Dead	6.25	5
	Normal	35.6	66.75
	Head	23.41	12.5
	Tail	31.22	14.5
	Double	3.52	1.25

Values M ± MSE. I: treated group; II: control group

Table (5) Serum levels of ALT, AST, and AP

Parameters	Groups		
	treated	control	Normal values
ALT u/l	26.4	31.4	10-35
AST u/l	28.4	24.85	0-40
AP u/l	74.8	103.4	35-129

Values M ± MSE. I: treated group II: control group

Discussion

The results revealed that the weight of sexual organs and the accessory glands, in addition to total sperm counts of treated males were less than those of control males, while the dead sperm and abnormalities in sperm parts was more in treated than in control one. The histological changes in sexual organs of exposed animals, might be attribute to many factors such as hormones (13). The number of dead sperm may be due to the spermicidal effects of the extract as (14) who proved its spermicidal effect on sperm in vitro. The decrease in sperm count in head of epididymis in male treated with melia could explain the decrease in testosterone hormone level as reported by many workers (15, 16) due to presence of receptors. According to (17) the addition of sodium nimibidinate salt in aqueous form to semen of rat and human results in death of sperm in different percentage. Neem oil claimed spermicidal activity against rhesus monkey, human spermatozoa in vitro, and when used in intra vaginally it prevents pregnancy in rats with concentration of 20 µl

and in rhesus monkey and women were about 10 ml. and the oral dose ads low as 25 µl prevents implantation in rats. According to (18), aqueous extract of old and tender leaves showed 100% of mortality of the sperm without altering its morphology (head, mid-piece and tail). The treatment with Melia azedarach in powder form in rabbits lead to occurrence significant changes in sex organs weights, and spermatogenesis, which might be attributed to many factors, the important one hormonal. The decrease in weight of epididymis and testes and the accessory organs might be attributed to ICSH and testosterone as the decrease in these two hormones leads to decrease in weight of sex organs and their functions (19). The reduced of pregnancy volume in female crossed with male treated with *Melia azedarach* can resulted from many variants in fertility the top of which is the reduced in sperm counts, increased dead sperm numbers and the percent of sperm deformity, in addition to disturbance of epididymis functions under the effects of androgen (20). The reduce in fertility also increased due to disturbances of functions of accessory sexual organs which supplied seminal plasma which is important for continuity of sperm life (20, 21). The increased in embryonic resorption and reduced weight of embryos might be attributed to sperm abnormalities, as many of these abnormalities could lead to inhibition of embryonic development, or might be due to deformity of endometrium functions before arriving of embryos (22). Conclusions: It was concluded from this study that *Melia azedarach* contained material of medical importance act as antifertility agent, through its action on number and morphology of sperms, meanwhile it has little side effects on health of animals.

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