Evaluation of antifertility effect and recovery of the seed oil constituents of Iranian species of Melia azadrach L. in male rats

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Abstract

Background: Convenient and effective contraceptive methods have been the subject of extensive and versatile research project, during the past 50 years. In this respect, the use of active herbal constituents is one of the topics of research and investigation.

Objective: In this study the antifertility activity of seed oil extract of Iranian species of Melia azadarach L. in northern district of country, on male rats, during to consecutive steps have been evaluated.

Methods: The seed oil extract have been prepared according to conventional methods, and were administered orally in 50 and 100 mg/kg daily doses for 60 days. In the first step, the inhibition of fertility indices were assessed with the help of, sperm motility, sperm viability, ESR(Epididymal sperm reserves), DSP(Daily sperm production), GSI(Gonado somatic index), fertility and testosterone concentration.
In the subsequent stage, 3 months after the 60th day of compounds administration, the reversibility of the a formentioned indices are determined again.

Results: In the first stage, a significant reduction in fertility indices to control especially in higher dose were observed. During the next stage, the significant increase in fertility indices are the indication of reasonable recovery and reversibility of extract activity.

Conclusion: In summary, the results of this study showed that its activity is reversible.

INTRODUCTION

In spite of considerable development in contraceptive technology, search for male antifertility agents continues to be a potential area of investigation [12,14].

Melia azaderach L. is a Iranian tree from the Meliaceae family that it found in north of Iran, from Lahijan to Gorgan[11]. The present study was undertaken to evaluate the effect of M. azaderach L. seed oil on fertility parameters of male rats, during consecutive steps.

MATERIALS AND METHODS

1- Animals: Adult male wistar rats(260 ± 20 gr body weight) were provided by the animal house of science faculty of Tehran university. Animals were maintained in plastic cages, under controlled temperature(25 ± 2º C) and light(12L , 12D).

2- Plant extract: seeds of M. azaderach L. are collected from Gorgan area and has been identified and deposited in the herbarium of pharmacognozy department of Tehran university (number of herbarium: 6640teh). Seeds were crushed and pulverized. 500 grams of the seeds powder were macerated with 1.5 litter of %70 Ethanol and was then filtered. The crude extract was obtained after removal of the solvent through vacuum distillation (60º C). Above process repeated for twice and extract oil was stored at 0'C.

3- Treatments: male rats of proven fertility were divided at random into 3 groups of 12 animals each. Group 1: Control animals received maize oil. Group 2: treated 50 mg/kg/day for 60 days. Groups 3: treated 100 mg/kg/day for 60 days. All animals received extract and maize oil through oral administration

4- Evaluation of parameters: Because that aim of this investigation was evaluation of antifertility effects and

recovery period, 24 hours after the last dose, animals of groups(n = 12) divided into 2 groups(n = 6).Study of fertility parameters in 2 stages were equal. First stage, 24 hours after last dose and second stage, 3 month after the last dose. In each stages, each male was caged separatedly with 2 coeval females of proven fertility in the evening for 6 days. Presence of sperms in the vaginal smears examined on the next day morning indicated that the females had mated to the particular male and the day of mating was taken to be day 1 of pregnancy[6]. In each stages after animals were sacrificed. Blood was collected by cardiac puncture and serum was separated. Reproductive organ (example: Epididymis weight and Testes weight) were dissected out and weighted and parameters studied:

Body weights of animals were recorded every week during treatment and before the experiments.

Sperm motility and sperm viability were assessed by the method of Prasad et al[8].

Epididymal sperm reserve (ESR) and daily sperm production(DSP) were assessed by method of Robb and et al[9].

Concentration of testosterone was determined by Radioimmunoassay (RIA)[10].

Fertility test was considered positive when implantation sites were present that was determinated by Oberlander and et al [7].

STATICAL ANALYSIS

The mean and standard error of mean (SEM) were calculated and significance of difference analyzed by applying student ?s ‘t' test.

RESULTS

Results of stage 1: the results summarized in table 1 and table 2. these tables indicated that a significant reduction was observed in GSI, sperm motility, sperm viability, ESR, DSP, testosterone concentration and fertility as compared with control group. It was also observated that high dose (100mg) has more significant difference as compared with low dose (50mg).

Figure 1

Table 1: Effects of extract oil on body and organs weight and motility and viability in first stage.

Group	Body weight (gr)	Epedadymis weight (1000 gr)	GSI (1000 gr)	Sperm motility (%)	Sperm viability (%)
control	64±2.64	66±0.47	7.66±0.31	78±0.56	81±2.58
Treated 1	75.5±6.64	61±0.36	6.35±0.09*	55±1.31***	59.5±2.45***
Treated 2	74.5±3.53	62.5±0.36	6.95±0.31*	46.75±1.31***	44±1.46***

n=6, Mean=±SEM, *p<0.05, **p<0.01, ***p<0.001

Figure 2

Table 2: Effects of extract oil on ESR, DSP, testosterone and fertility in first stage.

Group	ESR (Million)	DSP (Million)	Testosterone ConcentrationNg/dl)	Fertility (%)
control	224.4±3.78	25.63±1.42	591.5±38.23	79±4.83
Treated 1	197.52±4.01***	19.07±.58***	403±9.24**	45.5±1.43**
Treated 2	152.79±2.71***	12.94±0.66***	343.5±25.33***	30±9.25***

n=6, Mean=±SEM, *p<0.05, **p<0.01, ***p<0.001

Results of stage 2 : the results summarized in table 3 and table 4. these tables indicated that a significant reduction was observed in sperm motility, sperm viability, ESR, DSP and fertility as compared with control group.

Figure 3

Table 3: Effects of extract oil on body and organs weight and motility and viability in second stage.

Group	Body weight (gr)	Epedadymis weight (1000 gr)	GSI (1000 gr)	Sperm motility (%)	Sperm viability (%)
control	111.5±2.45	73.8±0.02	7.84±0.01	79±1.54	74±1.49
Treated 1	108±2.75	79.6±0.01	8.04±0.01	63.5±2.43***	64±1.84*
Treated 2	108.5±1.98	80.06±0.01	8.06±0.01	60.5±1.61***	60±1.06***

n=6, Mean=±SEM, *p<0.05, **p<0.01, ***p<0.001

Figure 4

Table 4: Effects of extract oil on ESR, DSP, testosterone and fertility in second stage.

Group	ESR (Million)	DSP (Million)	Testosterone ConcentrationNg/dl)	Fertility (%)
control	228.66±2	25.63±1.42	530±47.09	81.5±3.67
Treated 1	208.99±2.18***	17.28±1.12***	510.5±31.08	69.87±3.3
Treated 2	200.5±6.16***	16.03±0.71***	447.38.04	54.12±2**

n=6, Mean=±SEM, *p<0.05, **p<0.01, ***p<0.001

Results of recovery period: the results summarized in table 5 and table 6. these tables indicated that a significant increase was observed in sperm motility, sperm viability, ESR, DSP, testosterone concentration and fertility as compared with stage 1. Fertility in stage 1 and stage 2 observed in figure 1.

Figure 5

Table 5: Compare of GSI, motility and viability in first stage with second stage.

Group	stage	GSI 1000.gr	Sperm motility (%)	Sperm viability %
Treated 1	First	6.35±0.09	55±1.31	59.5±2.45
	Second	8.04±0.01***	63.5±2.43	64±1.84*
Treated 2	First	6.95±0.31	46.75±1.31	44±1.46
	second	8.06±0.01*	60.5±1.61*	60±1.06**

n=6, Mean=±SEM, *p<0.05, **p<0.01, ***p<0.001

Figure 6

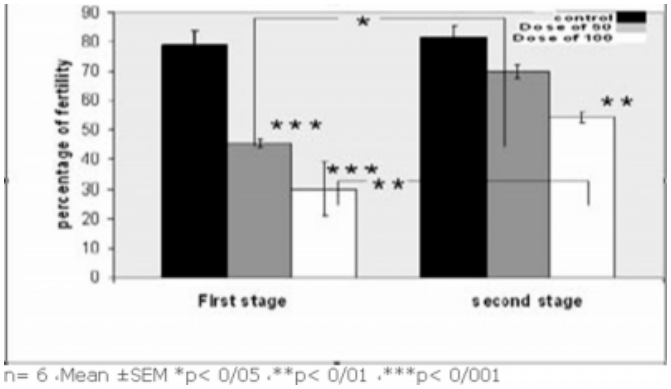
Table 6: Compare of ESR, DSP, testosterone and fertility in first stage with second stage.

Group	stage	ESR (Million)	DSP/g t (Million)	Testosterone Concentration(Ng/dl)	Fertility (%)
Treated 1	First	197.52±4.01	19.07± 58	403±9.24	45.5±1.43
	Second	208.99±2.18*	17.28±1.12	510.5±31.08*	69.87±3.3*
Treated 2	First	152.79±2.71	12.94±0.66	343.5±25.33	30±9.25
	second	200.5±6.16***	16.03±0.71*	447.38.04*	54.12±2**

n=6, Mean=±SEM, *p<0.05, **p<0.01, ***p<0.001

Figure 7

Figure 1: Percentage of fertility in stage 1 and stage 2



n= 6 ،Mean ±SEM *p< 0/05 ،**p< 0/01 ،***p< 0/001

DISCUSSION

Results of this investigation indicated that oral administration of seed oil of M. azaderach in particular with high dose, cause a significant decrease in fertility parameters. In the present study, 3 month after the last dose observed a significant increase in fertility parameters and it indicated that its antifertility is reversible.

Similar results were found by the administration of Alstonia scholaris in rats, Mentha arvensis in mice, Terminalia bellirica extract on rats, Cleistanthus collinus on reproductive parameters and Piper betle on rats[1,3,14] and etc[2,3,4,5].

Reduction in fertility parameters may be due to altered androgenic synthesis and spermatogenesis[6,13].

In summary, this study showed that administration of this extract has antifertility effect but its activity is not constant.

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