

The negative effect of flaxseed oil on reproduction through its effect on spermatogenesis and ovulation in mice

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ABSTRACT

Objectives: Infertility is defined as failure to achieve pregnancy after 12 months of sexual intercourse without any contraceptive measures. Flaxseed oil has broad spectrum uses in health aspects. It has been getting a special attention in treatment of infertility. The aim of our study is to evaluate the effect of flaxseed oil on fertility in mice

Methods: The study included seventy-five mice (Bulbe C). Each gender of mice was randomly divided into three groups, one group serves as control group while the other two groups were treated with flaxseed oil extract with either a dose of 60 mg /Kg BW/ day or a dose of 120 mg /Kg BW/ day for 2 weeks. At the end of treatment period the animals were redistributed in a way that each female group was subjected to mate with male mice from the three male groups to study the reproductive efficiency alteration by flaxseed oil extract.

Results: There was no pregnancy in all groups treated with flaxseed oil and azoospermia in all treated male groups with serious histological changes in testes and epididymis.

Conclusion: There was obvious negative effect for flaxseed oil on the reproduction in male and female mice regarding pregnancy percentage, sperm count and histological changes of testis and epididymis.

Keywords: Fertility, infertility, sperm count, pregnancy, flaxseed oil

INTRODUCTION

Infertility is defined as failure to achieve pregnancy after 12 months of sexual intercourse without any contraceptive measures [1]. It is a wide spread medical problem that affects millions of peoples over the world [2]. The causes of infertility in males differ from those in females. In males, infertility is most commonly due to varicocele, asthenozoospermia and oligospermia. While in females, it is usually due to polycystic ovary syndrome (PCOS), pelvic inflammatory diseases (PID) or diseases of the fallopian tubes [3]. Flaxseed oil is an oil rich with alpha linolenic acid [4]. It has broad spectrum uses in health aspects [5,7]. It has been getting a special attention in treatment of infertility [8,9].

The dietary habits affect reproductive life [10,11]. Some studies suggest that the flaxseed oil may improve reproductive health by affecting hormonal balance, anti-inflammatory effect, improving sperm quality and motility, and improve ovulation [8,9]. Flaxseed oil also has antioxidant properties protecting reproductive cells from oxidative stress [12]. However, some studies concluded harmful effects for flaxseed oil [13,14]. The aim of our study is to evaluate the effect of flaxseed oil on fertility in mice as mice are considered the commonly used model around the world because their reproductive cycle is short and they are close to humans physiologically [15].

MATERIAL AND METHODS

Study design:

The study included seventy-five mice (Bulbe C) from animal house where they provided with ad libitum feeding. Each gender of mice was randomly divided into three groups as following

Male groups:

Control group: includes 10 males

Group1: includes 10 males treated with oral dose of flaxseed oil 60 mg /Kg BW/ day for 2 weeks

Group 2: includes 10 males treated with oral dose of flaxseed oil 120 mg /Kg BW/ day for 2 weeks

Female groups:

Control group: includes 15 females

Group 1: includes 15 females treated with oral dose of flaxseed oil 60 mg /Kg BW/ day for 2 weeks

Group 2: includes 15 females treated with oral dose of flaxseed oil 120 mg /Kg BW /day for 2 weeks

At the end of treatment period the animals were redistributed in a way that each female group was subjected to mate with male mice from the three male groups to study the reproductive efficiency alteration by flaxseed oil extract.

Preparation of flaxseed oil:

The extraction of flaxseed oil was prepared for this study by grinding the clean seeds of flaxseed to increase the surface area for extraction then the oil was obtained by using normal hexane as solvent in soxhlet apparatus, then the hexane was evaporated and the flaxseed condensed oil was obtained. Then it was kept in dark container to maintain the flaxseed oil stability. [16]

Reproductive efficiency assessment:

1-Percentage of pregnancy

Obtained by divided the number of pregnant females on the total number of females in that group

2-Sperm count

The sperms were counted according to method of Evan and Maxwell (1987) by using Neubauer hemocytometer chamber that is used for RBC and WBC count [17].

3-Histological study

Sorted fragments of testes and epididymis were collected from all groups at the end of period of mating and prepared for histological study according to Mescher method (2018) with aid of the light microscope [18].

RESULTS

Table (1) shows the comparison in pregnancy percentage between control group and treated groups after a period of 4 weeks for mating. There was 80% pregnancy in the control female group while it was zero in all groups treated with flaxseed oil.

The sperm count of control group was 168 million/ml of semen from epididymis while it was zero in all treated male groups as shown in table (2)

Histological findings:

1-Testis

Histological examination of the testes shows irregular shape or atrophied seminiferous tubules with suppression of spermatogenesis in mice treated with flaxseed oil 60 mg /Kg BW/ day for 2 weeks as shown in figure (1), while there was excessive suppression of spermatogenesis and necrosis of supporting cells in male mice treated with flaxseed oil 120 mg /Kg BW/ day for 2 weeks as shown in figure (2).

2-Epididymis

Histological examination of the epididymis of male mice treated with flaxseed oil 60 mg /Kg BW/ day for 2 weeks shows haemorrhage in interstitial tissue and vacuolation of epithelial cells as shown in figure (3). In addition to that thickening of capsule was obvious in male mice treated with flaxseed oil 120 mg /Kg BW/ day for 2 weeks as shown in figure (4).

DISCUSSION

Many studies confirm the benefits of flaxseed oil for reproduction through its effect on spermatogenesis, ovulation and hormonal balance [19,20]. However, the sperms count in our study was completely diminished by the administration of flaxseed oil. This result could be attributed to the use of high dose of flaxseed oil. flaxseed oil contains phytoestrogens [21]. Phytoestrogens affect the function of the male reproductive system [22]. They bind to estrogen receptor alpha (ER α) and estrogen receptor beta (Er β) because they mimic the structure of estradiol [23]. Estradiol inhibits the proliferation of Leydig cells. It impairs regeneration of Leydig cells and inhibits testosterone biosynthesis enzymes causing testosterone deficiency [24]. Testosterone play essential role in sperm production and its deficiency results in reduced sperm counts [25]. In addition, estrogen-like substances inhibit the proliferation of Sertoli cell and affect spermatogenesis process [26].

Histological examination reveals that flaxseed oils consumption induced histopathological changes in the testes like irregular shape or atrophied seminiferous tubules with suppression of spermatogenesis (as shown in figure 1 and 2) and histopathological changes in the epididymis in form of vacuolation of epithelial cells, thickening of capsule and hemorrhage in interstitial tissue (as shown in figure 3 and 4). Our findings are similar to those reported by Helal et al [27].

Although there were several studies concluded that flaxseed oil improves ovulation [8,20,28], our study shows no pregnancy in treated female mice even when mated with non-treated male mice as shown in table [2]. The zero percentage of pregnancy may be caused by several mechanisms: First mechanism, flaxseed contain large amount of lignans [29]. Lignans have potent anti-estrogenic properties. It can modify enzymatic activity,

estrogen synthesis and receptor activity [30,31]. Second mechanism, flaxseed decreases prostaglandin E2 [PGE2] [32]. PGE2 is an important regulatory prostaglandin that regulates ovulatory processes, follicular wall luteinization and the release of ovarian oocytes [33]. Third effect, omega 3 fatty acids and lignans enhance apoptosis of ovarian cells [34]. Forth effect, flaxseed negatively affects the sperm penetration rate [35].

CONCLUSION

Flaxseed oil extract showed obvious negative effect on reproduction in male and female mice regarding pregnancy percentage, sperm count and histological changes of testis and epididymis, when ingested for two weeks with 60 or 120 mg/Kg B.W

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Table 1: Pregnancy percentage among female groups after being subjected to mate with male mice from the three male groups to study the reproductive efficiency alteration by flaxseed oil extract.

Group	Pregnancy %
Control M + Control F	80%
Control M+ G1 F	0%
Control M+ G2 F	0%
Control F+ G1 M	0%
Control F+ G2 M	0%
G1 M + G1 F	0%
G1 M + G2 F	0%

G2 M + G1 F	0%
G2 M + G2 F	0%

Control M: Control male group

Control F: Control female group

G1 F: Group 1 female treated with oral dose of flaxseed oil 60 mg /Kg BW/ day for 2 weeks

G2 F: Group 2 female treated with oral dose of flaxseed oil 120 mg /Kg BW/ day for 2 weeks

G1 M: Group 1 male treated with oral dose of flaxseed oil 60 mg /Kg BW/ day for 2 weeks

G2 M: Group 2 male treated with oral dose of flaxseed oil 120 mg /Kg BW/ day for 2 weeks

Table 2: Sperm count for male groups:

Group	NO. of sperm in million /ml of semen [mean \pm SD]
Control group [N=10]	168 \pm 5.17
G1 [N=10]	0
G2 [N=10]	0

G1: Group 1 includes 10 males treated with oral dose of flaxseed oil 60 mg /Kg BW/ day for 2 weeks

G2: Group 2 includes 10 males treated with oral dose of flaxseed oil 120 mg /Kg BW/ day for 2 weeks