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Effect of *Azadirachta excelsa* (Jack) Leaf Extracts on the Reproductive Organs and Fertility of Male albino Mice (*Mus musculus*)

Waad S. Shaher
Department of Biology
College of Science
Mosul University

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ABSTRACT

This study was conducted to investigate the effect of oral dose (250mg/kg body weight/day, for 21 days) of each aqueous and alcohol leaf extract of *Azadirachta excelsa* on histological structure of the testis and fertility of male albino mice *Mus musculus*. Histological structure of the testis of both treated groups showed affected seminiferous tubules indicating mixing of the germ cell types in stages of spermatogenesis, atrophy of the spermatogenic elements, increases in number of Leydig cells, occurrence of giant cells and decrease s or absence of the spermatozoa in the lumen of the seminiferous tubules as compared with control group. The other alternations of both treated groups were decrease in number of the spermatozoa in the Ductus epididymidis. The fertility index of the treated groups was reduced, this result which proves the fertility was observed in untreated females after mated with treated males.

Keyword: *Azadirachta excelsa*, Leaf extract, Testis, Histopathology, Spermatozoa, Fertility.

تأثير مستخلصات اوراق *Azadirachta excelsa* على الاعضاء التكاثرية وخصوبة ذكور الفئران البيض السويسرية *Mus Musculus*

الملخص

اجريت هذه الدراسة لملاحظة تأثير الجرعة الفمية (250 ملغم/كغم من وزن الجسم لمدة 21 يوما) لكل من المستخلصين المائي والكحولي لاوراق نبات *Azadirachta excelsa* على التركيب النسجي للخصى والخصوبة لذكور الفئران البيض السويسرية *Mus musculus*. التركيب النسجي في الخصية لكلا المجاميع المعاملة اظهر نبيبات منوية متأثرة تضمنت مزج وعدم انتظام انواع الخلايا الجرثومية في مراحل تكوين النطاف، وضمور العناصر المنطفة، وازدياد في اعداد خلايا ليدج، وظهور خلايا عملاقة، وانخفاض او

غياب النطاف في تجويف النبيبات المنوية مقارنة بمجموعة السيطرة. ومن التغييرات الاخرى لكلا المجاميع المعاملة هو انخفاض اعداد النطاف في نبيبات القناة البربخية. ووجد اختزال في مقياس الخصوبة في المجاميع المعاملة وهذه النتيجة التي تبرهن الخصوبة لوحظت في الاناث غير المعاملة بعد تزاوجها مع الذكور المعاملة.

INTRODUCTION

Azadirachta excelsa belongs to family meliaceae, which comprises of 50-52 genera with about 550 species, it looks like a very tall Neem tree (*A.indica*) and it may reaches a height of 40-50 meters, it is known by different common names, one of these Marrango in Philippine (Schumutterer and Doll, 1993; Norani, 1997). The highest yields of azadirachtin of *A.excelsa* leaf extracts were not restricted to species provenance but they came from single tree of different origin (Nor Aini and Seong, 2006).

Meliaceae is a plant family whose tree has numerous useful characteristics such as medical and pesticidal properties. One candidate of this family, *Azadirachta indica*, possesses these characteristics and has been used widely in ayurvedic medicine in India (Randhawa and Parmar, 1993). Also *A.excelsa* tree with various parts have been used as traditional medicine, (antiseptic, anti-inflammatory, antimicrobial agent) antifeedant, insecticidal (Chungponse and Buranatham, 1991; Lean *et al*., 2003; Akhatar *et al*., 2008). Macedo *et al*., 2007, observed that the increasing doses of *A.indica* leaf extract did not improve the control of endoparasites naturally infected.

Numerous studies on experimental animals have been shown that *A.indica* seed extracts have transient and reversible antifertility and abortive effects (Bardhan *et al*., 1991; Mukherjee *et al*., 1999). Antifertility effects of neem oil were observed by Shakati *et al*., 1990, in female rats which remained infertile for variable periods.

There are a few studies about the effects of *A.excelsa* on mammals, one of these studies referred that in concentrations up to 200ppm, the leaf extracts of *A.excelsa* was much more effective than of *Melia azadarach*, the extract of *A.indica* was in the third place (Doll and Schumutterer, 1993; Schumutterer and Doll, 1993; Hein, 1994).

The objective of this study was to determine the effect of *A.excelsa* leaf extracts on reproductive organs and fertility in male mice.

MATERIAL AND METHODS

Plants:

Green leaves of *Azadirachta excelsa* J. were collected from the gardens of university of Mosul during August 2005. Leaves washed in running tap water and dried to powder using a mechanical grinder.

Preparation of extracts:

The method of plant extraction was modified from (Choochote *et al*., 1999). 50grams of the powdered leaves was macerated with 200ml of 75% ethanol alcoholic solution and left to stand at room temperature for 24 hours. The mixture was filtered through a Whatman

no.1 filter paper by suction and the filterate was evaporated under vaccum at 40°C until completely dried and kept at constant 4°C until needed for experiments. For aqueous extract 50g of the powdered leaves was macerated with 200ml of distilled water for 24 hours and the mixture was filtered.

Animals:

In this study 15 males, 18 females of albino mice were used. They were 3 months old, weighting (22-28g). The males were divided into 3 groups each one contains 5 males isolated in plastic cages. The first group was given 0.2ml d.w. and regarded a control group. The second group was forced feeding orally dose of aqueous extract (250mg/kg) of body weight daily for 3 weeks. The third group with alcohol extract (250mg/kg) of b.w. daily for the same period. All groups were exposed to a constant laboratory condition, temperature was about 25°C and light/dark cycle of 12:12 h. and fed with standard commercial diet and given water.

Three of treated males of each group were mated with six untreated nonpregnant females to prove fertility (one male with two females). After one week the males were removed from the cages. The pregnant females were observed daily after 3 weeks of gestation and the number of offspring from each female was recorded and weighted as soon as possible after birth. The other two males of each group were anesthetized with chloroform and dissected, testis were carefully isolated for study of histopathological changes. After routine preparations the organs were embedded in paraffin and sections were cut 6µ and stained with a double stain haematoxylin-eosin.

RESULTS

The effects of oral administration (250mg/kg body weight/day for 21 days) of aqueous and alcohol leaf extracts of *Azadirachta excelsa* on male reproductive organs and fertility of male albino mice were investigated. The section of the testis in control group indicating normal spermatogenesis (Fig. 1), the seminiferous tubules were lined with three or four regular layers of seprmatogenic cells at different stages of maturation, and mature spermatozoa were observed. But in the treated mice, the testes showed affected seminiferous tubules, indicating mixing of the germ cell types in the stages of spermatogenesis, mass atrophy of spermatogenic elements, degenerated appearance of germ cells and decreased or absence of the spermatozoa in the lumen of seminiferous tubules (Fig. 2, 3).

The affected seminiferous tubules showed loosening some of the germinal epithelium and separating the spermatogenic cells from the germinal layer (Fig. 2), and increase in number of Leydig cells (Fig. 4, 5).

In both treated groups, seminiferous tubules showed congestion of the blood vessels, and occurrence of giant cells (Fig. 6, 7). A general reduction in diameters of some seminiferous tubules of alcohol treated group and foamy substance has been observed as well as a reduction of interstitial tissues. (Fig. 8).

The treated groups also showed a general decrease in the number of spermatozoa in the lumen of the ductus epididymidis as compared with control group (Fig. 9, 10, 11). Also alcohol treated group showed a reduction in diameters of the ductus epididymidis (Fig. 11).

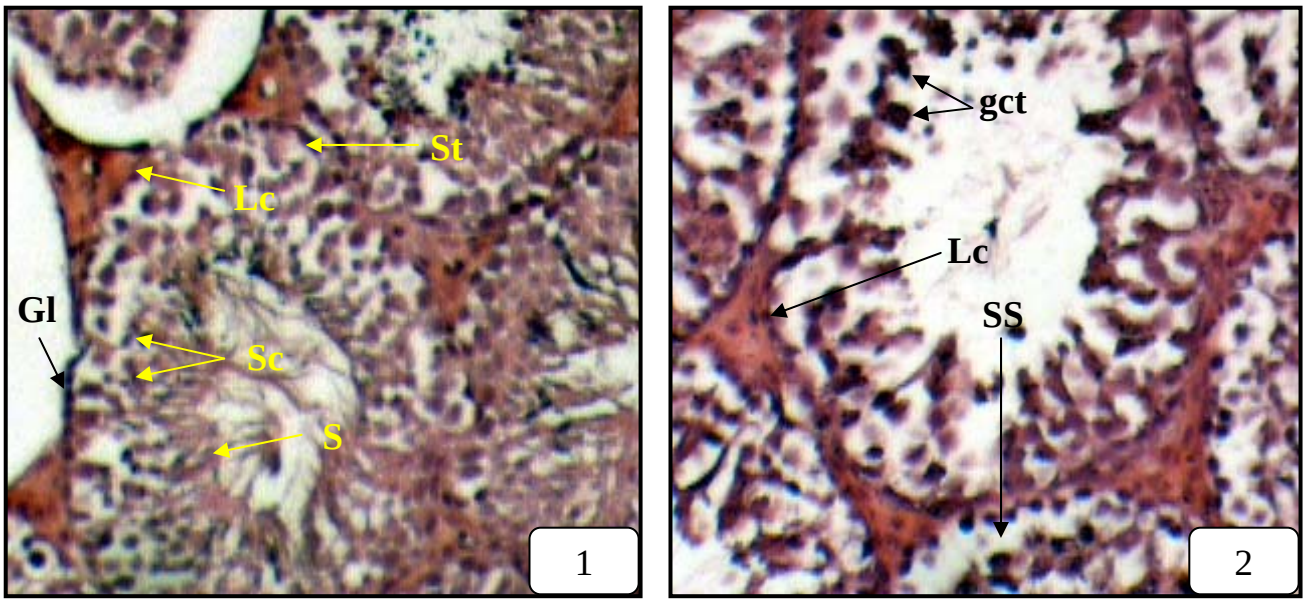


Fig.1: Section of the testis of the control male mice, *Mus musculus* showing Seminiferous tubules-St, with (3-4) Layers of spermatogenic cells-Sc, spermatozoa-S, germinal layer-Gl, Leydig cells-Lc. H.and E. X160.

Fig.2: Section of the testis of the male mice treated with aqueous leaf extract of *A.excelsa* showing mixing of germ cell types-gct, absence of spermatozoa, occurrence of separating space-SS, Leydig cells-Lc, H.and E. X160.

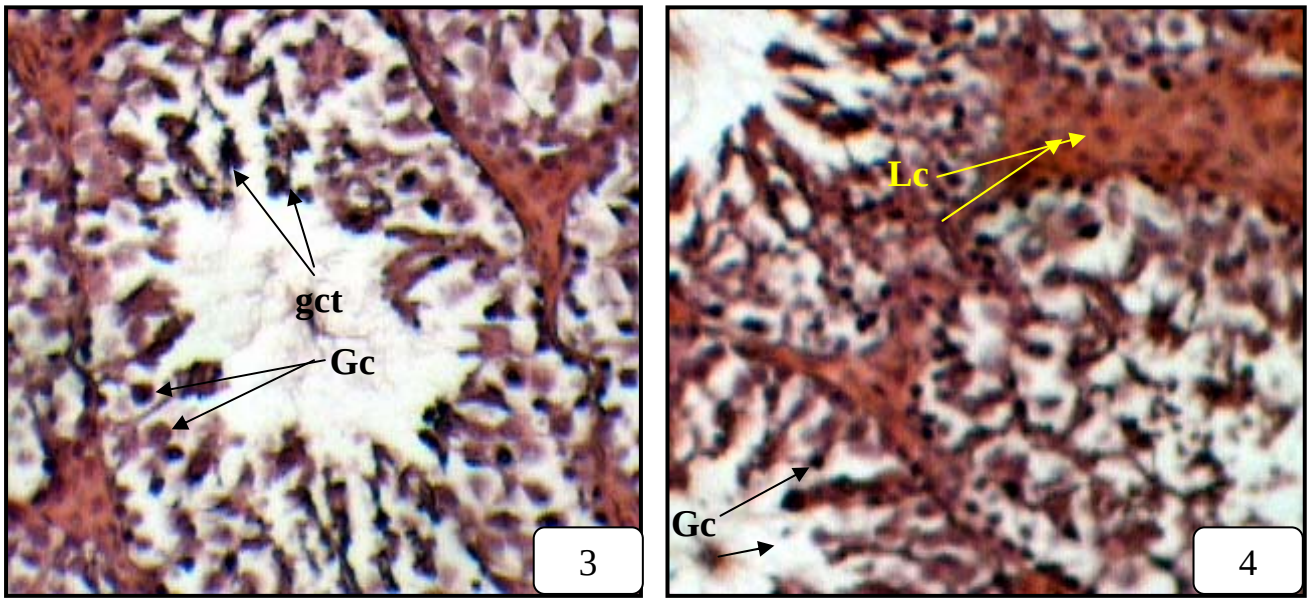
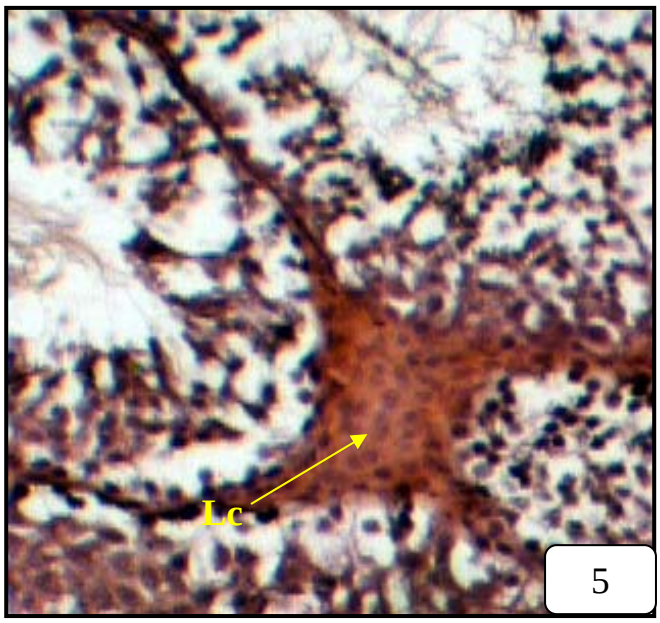


Fig.3: Section of the testis of male mice, treated with alcohol leaf extract of *A.excelsa* showing mixing of germ cell type-gct, absence of spermatozoa in seminiferous tubules. Occurrence giant cells- Gc. H.and E. X160.

Fig.4: Section of the testis of the male mice treated with aqueous leaf extract of *A.excelsa* showing increasing of Leydig cells -Lc, occurrence of giant cells-Gc. H. and E. X160.



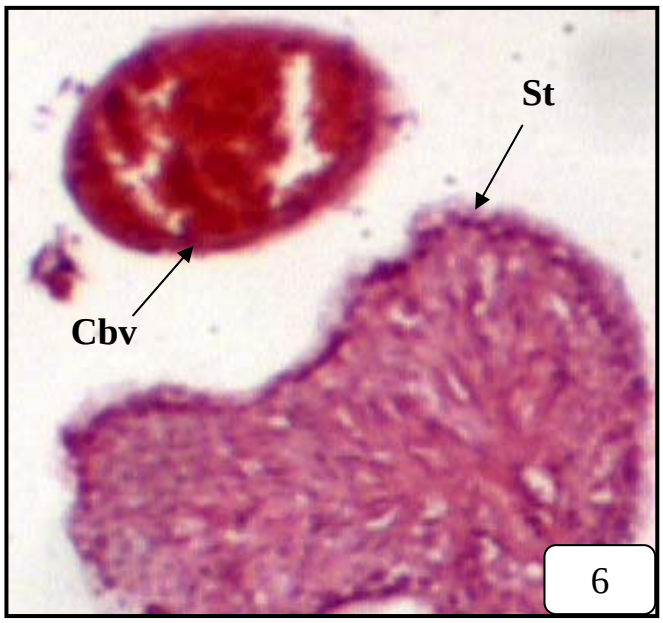
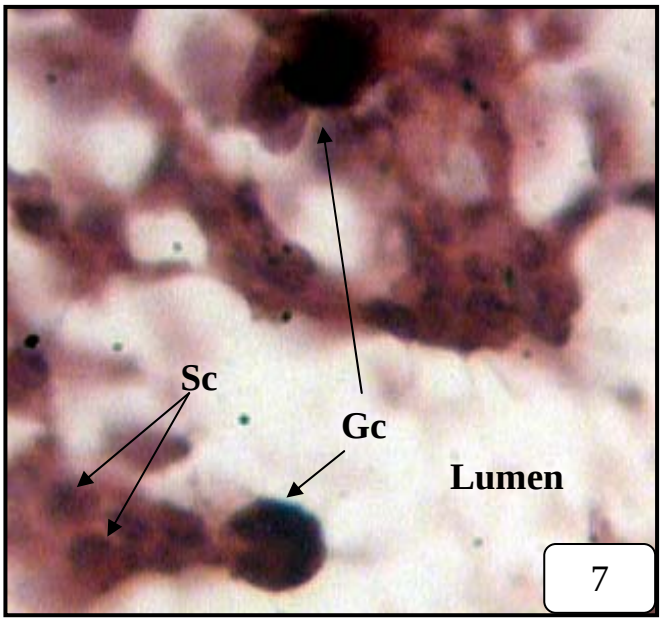


Fig.5: Section of the testis of male mice, treated with alcohol leaf extract showing increasing Leydig cells-Lc. H.and E. X160.

Fig.6: Section of the testis of the male mice treated with aqueous leaf extract showing congestion of blood vessel-Cbv, Seminiferous tubule-St. H.and E. X640.



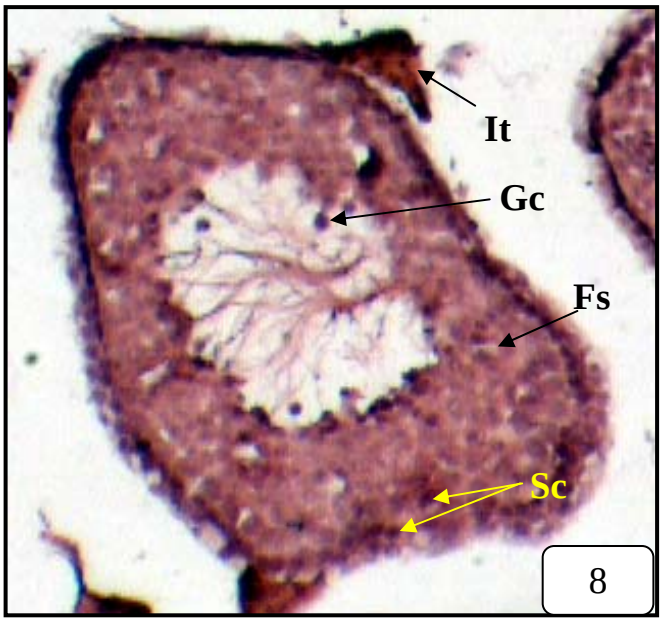


Fig.7: Section of the testis of male mice, treated with aqueous leaf extract showing occurrence of giant cells-Gc, spermatogenic cells-Sc. H.and E. X640.

Fig.8: Section of the testis of the male mice treated with alcohol leaf extract showing a reduction in diameters of seminiferous tubule mass atrophy of spermatogenic elements and foamy substance-Fs, interstitial tissues-It. H.and E. X160.

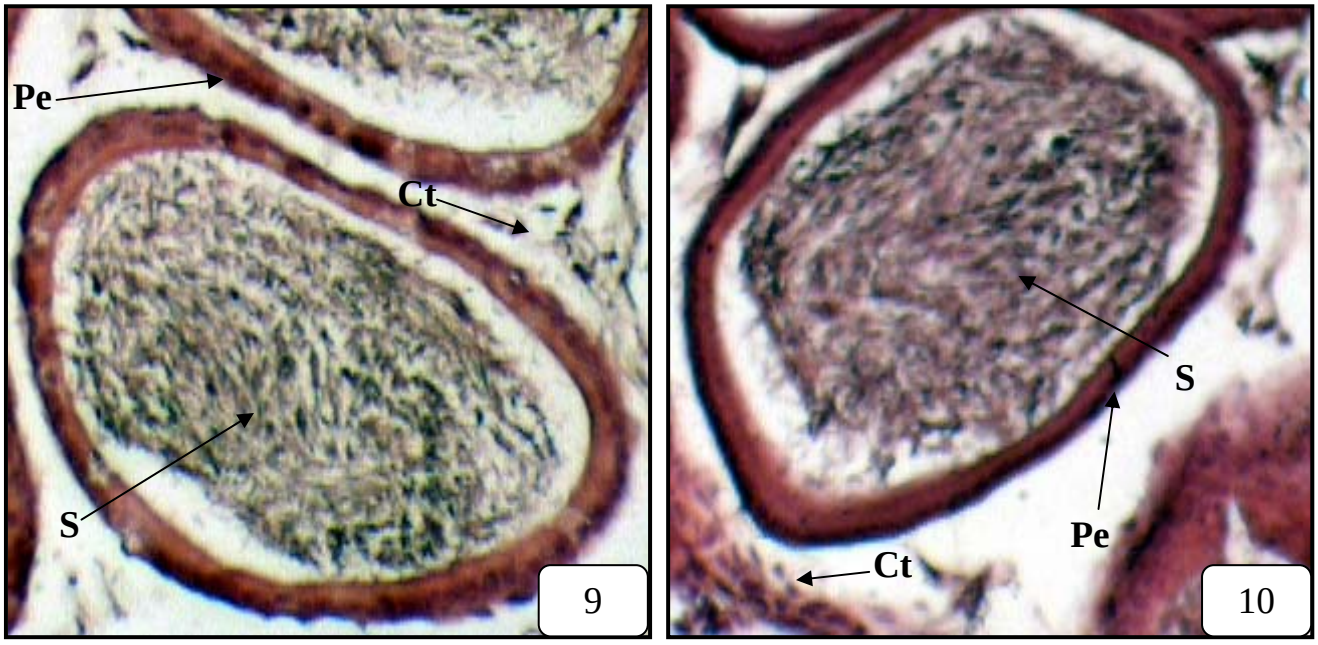


Fig.9: Section of the ductus epididymidis of control male mice, showing pseudostratified epithelium-Pe, lining the duct. Large mass of the sperms-S, in the lumen of the ductus connective tissues-Ct. H.and E. X160.

Fig.10: Section of the ductus epididymidis of the a queous treated group showing a less mass of sperms-S in the lumen of the ductus as compared with control group. H.and E. X160.

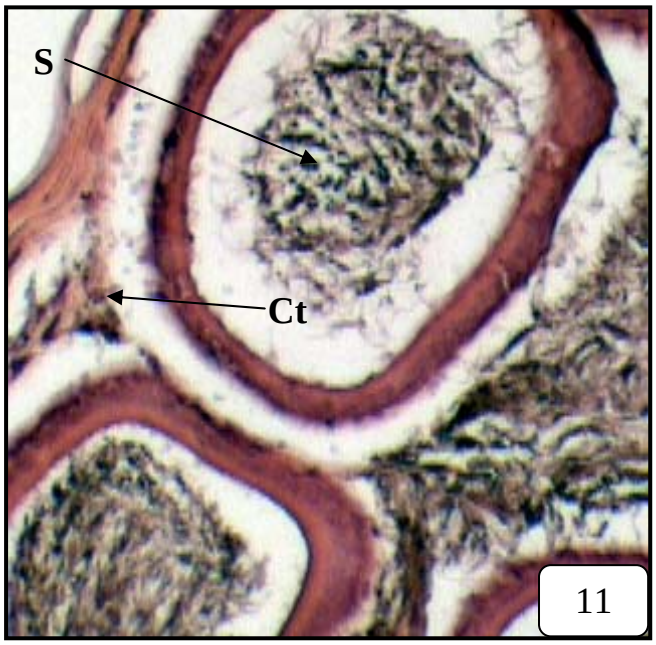


Fig.11: Section of the ductus epididymidis of the alcohol treated group showing a least mass of the sperms-S, in the lumen of the ductus as compared with control group, connective tissue-Ct. H.and E. X160.

The results showed that the mean number of newborns was decreased in the normal females which mated with treated males and the fertility index was reduced from 100% in control group to 83.33%, 66.66% in aqueous and alcohol treated groups respectively (Table 1).

Table 1: Effect of *Azadirachta excelsa* leaf extracts on mean number of newborn and fertility index (six females for group).

Group	**Dose mg/kg**	**No. of females pregnant**	**Mean No. of newborn±SE**	**Fertility %**
Control	-	6	8.0±0.32	100
Aqueous *A.excelsa* extract	250	5	5.0±2.61	83.33
Alcohol *A.excelsa* extract	250	4	3.0±2.28	66.66

DISCUSSION

In this research the effects of aqueous and alcohol leaf extract of *A.excelsa* were studied using histopathological examination of testes and fertility index.

In this Study the affected Seminferous tubules showed separating of the spermatogenic cells from the germinal layer (Fig.2), this due to the germ cells were not produced primary spermatocytes. In addition congestion of blood vessels, this effect was occurred as a result of defection in blood flow to the main veins. The increasing in Leydig cells is leading to increase testosterone, and this hormone excites epithelial tissue of semniferous tubules to products sperms. The results observed occurrence of giant cells which appeared in many histopathological alteration, this is in agreement with Mishra and Singh, (2005).

It can be seen from the results that *A.excelsa* leaf extract possesses male antifertility agent in mice which was found to be reversible on fertility, (Table 1). The result indicated that male antifertility due to the effect of the extract on the different types of germ cells, perhaps associate with hormonal effect on spermatogenesis. This result is in agreement with Sadre *et al*., (1983), who reported that *A.indica* aqueous leaf extract caused antifertility activity in rats. Also Mishra and Singh (2005) observed that *A.indica* leaf extract caused reversible alternations in the reproductive organs of mice.

In this study, the results showed a decrease in spermatozoa in treated groups compared with control group (Fig. 2, 3), thus the affected sprematogenic cells do not reach their normal maturation, each of these effect on any stage leads to the retardation of spermatogenesis in males. Azadiractin has the ability to act as a powerful spermicidal, Sadre *et al*., (1983). Kasturi *et al*., (2002) found that Crude leaf extract of *A.indica* caused a defects in late spermatids and other effects in spermatogenesis in albino rats through antispermatogenic and antiandrogenic properties.

In the present study, the results showed that mean number of newborns was decreased in females which mated with treated male groups. Fertility index was reduced by the effect

of *A.excelsa* extracts on the number of spermatozoa and its ability to fertilize. Sadre *et al*., (1983) reported that *A.indica* leaf extract caused reduction in fertility and decreased the mean number of newborn in rats. Female rats were remained infertile for variable periods ranging from 107 to 180 day after given a single dose (100µl) of neem oil by intrauterine route (Shakti *et al*., 1990). Shahir (2002) also found the same effects of crushed fruits of *Melia azedarach* in rats.

In addition, *Ailanthus excelsa* leaf extract and stem bark at dose 250mg/kg b.w. exhibited a remarkable antimplantation and early abortificient activity in female albino rats (Dhanashekaran *et al*., (1993), Morovati *et al*., (2008)) reported that *A.indica* extract Neem Azol-T/s caused increase in mean delay for pregnancy in rats.

From this results *A.excelsa* extracts could be used as an antifertility agent to control harmful agricultural rodents.

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