

Original Research Article

Effects of Almond Seed Oil Extraction and Some Antioxidant Agents on Sperm Quality in Alloxan-Induced Diabetes Mellitus Rat

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A B S T R A C T

This study aimed to examine the result of Effects of almond oil, vitamin E, L-carnitine and vitamin E + L-carnitine on sperm quality in alloxan induced diabetic rats. Ninety male albino rats weighing between 200 – 250 g, have been utilized. The animals housed under standard laboratory conditions (12 h light: 12 h dark photoperiod), 22± 2 C°, and the animals were given standard rat pellets and tap water ad libitum. Diabetes was induced experimentally by a single subcutaneous injection of rats with diabetogenic agent alloxan (120 mg/kg). After four weeks, rats with blood glucose more than 200 mg/dl were considered as diabetes. The animals arbitrarily divided into six groups, first group regarded as normal control rats, second group considered as diabetic control rats, third group treated with vitamin E (2000 IU/kg diet), fourth group treated with L-carnitine, L-carnitine (5gm/kg diet), fifth group treated with vitamin E (2000 IU/kg diet) + L-carnitine (5gm/kg diet) and the sixth group treated with Almond oil treated diabetic group. Rats of the sixth group received 1 ml/kg BW of almond oil per rat per day, for five weeks. The oil mixed with the diet. At the end of the treatment, the result showed the levels of Sperm Count, Sperm live percent, sperm motile percent, and sperm healthy morphology percent were significantly ($P \leq 0.05$) decreased in diabetic rats fed with regular diet.

Keywords

Almond oil extraction,
Diabetes mellitus,
Sperm quality,
Alloxan induced rats,
Antioxidant

Introduction

Introduction Diabetes mellitus (DM) is a condition with high frequency worldwide thus the disease constitutes a major health concern. Presently, it is an incurable metabolic disorder that affects about 2.8% of the global population (Etuk, 2010). The frequency of diabetes has been rising rapidly from 135 million in 1995 to an estimated 380 million in 2025 (Vassort and Turan,

2010). It has shown about 90% of diabetic patient suffer from a deficiency in sexual disturbance including libido and fertility (Scarano et al., 2006).

The 90% of the male diabetic patients experiencing less sexual functionality, Animal model that induced diabetes provide a relevant model to study reproductive

dysfunction because they show evidence of number of deficits in generative role that look like those seen in human diabetics (Steger et al., 1989) also reduced semen quality has correspondingly been reported in diabetic men, involving decreased sperm motility and concentration, abnormal morphology and increased seminal plasma abnormalities (Amaral et al., 2008).

Prunus amygdalus belongs to the Rosacea family, and is a medicinal plant popularly used in the treatment of several diseases (Shah et al., 2011). Almonds contains high levels of fiber, arginine, magnesium, polyphenolic compounds, vitamin E, and monounsaturated fatty acids (MUFA), and consumption of more nut and peanut butter lowering risk of type 2 diabetes (Jiang et al. 2011).

Materials and Methods

Adult male laboratory rat used in the present study, (8-10 weeks) in age, weighing (200-250) gm, kept in the animal house at the Department of Biology, Faculty of Science and Education Science, Sulaimani University/Iraqi Kurdistan- Region, in precise environment that was maintained under a 12 hour light/dark cycle, a temperature of 22 ± 2 °C and The rats supplied with a standard pellet diet and water ad-libitum (Abo-Ghanema et al., 2012).

Induction of diabetes

After fasting overnight (access to water only) the diabetes was induced in male rats were given a single S.C injection of freshly prepared alloxan monohydrate (BDH Chemical Ltd.) (120 mg/kg of body weight) solution using saline (0.9% (w/v)). The control animals received saline vehicle only (Bahnak and Gold, 1982). Alloxan injected rats were given 5% glucose overnight to

prevent rapid fatal hypoglycemia resulted from insulin release due to alloxan action

Experimental design

Thirty-eight (30) adult male rats used in the current study, after one month of induction of diabetes mellitus, they were separated into two main groups; diabetic and non-diabetic (control) groups. Diabetic group divided into five subgroups (Table1).

At the end of each experimental period, (5 weeks), blood samples were collected, from fasted rats (control and treat animals), using anesthetic with ketamine hydrochloride (50mg/ Kg b.w.) (Alp et al., 2012) and sacrificed, heart puncture took blood sample. Put into chilled tubes without EDTA for serum collection (biochemical test); later centrifuged at 3000 rpm for 15 minutes at 4°C then serum stored in Deep Freeze (-45 C°).

Then epididymis, were surgically removed, the cauda epididymides of both sides separated from tests and put into small Petri containing one ml of Dulbecco medium, (Seed et al., 1996) pre-warmed to 37 °C, cauda epididymides was cutting into several parts by a sharp microsurgical scissor in order to release the sperm into solution Attempts were made to remain the temperature of the Instruments and liquid at about 37°C during the sperm collection and analysis (Hosseini et al., 2012; El- Kashoury et al., 2010). The following parameters were measured. The semen sample was analyzed for its count, motility, viability, and morphology. Semenology performed as per guidelines of (WHO, 2010).

Statistical analysis

Analysis of data was performed by using SPSS (Version 18). Results expressed as mean \pm S.E. Statistical differences were

determined by Dunnett's test for multiple comparisons after ANOVA. Dunnett test treats one group as a control and compares all other groups against it.

Results and Discussion

Sperm count in diabetic rats significantly ($P<0.05$) reduced ($19.25 \pm 1.25 \times 10^6/\text{ml}$) when compared to control rats ($36 \pm 2.768 \times 10^6/\text{ml}$).

Treatment of diabetic rats with almond oil, L-carnitine, and (L-carnitine+Vitamin-E), significantly ($P<0.05$) elevated sperm count ($31.00 \pm 3.363 \times 10^6/\text{ml}$, $31.20 \pm 4.042 \times 10^6/\text{ml}$, $32.20 \pm 0.860 \times 10^6/\text{ml}$) respectively in comparison with untreated diabetic rat group ($19.25 \pm 1.250 \times 10^6/\text{ml}$). However, diabetic rat treated with vitamin-E showed no significant change ($25 \pm 1.303 \times 10^6/\text{ml}$) in comparison with untreated diabetic rat group (Table 4.1 and Figure 4.1).

Sperm viability

Sperm live percent in untreated diabetic rat group significantly ($p<0.05$) reduced ($59.645 \pm 1.250 \%$) when compared to control rat group ($85.088 \pm 0.758 \%$). Diabetic rat group were treated with almond oil, vitamin-E, L-carnitine, and (vitamin-E + L-carnitine) showed significant ($p<0.05$) increase in the percentage of live sperm ($75.200 \pm 0.786\%$, $74.398 \pm 0.675\%$, $72.332 \pm 0.794\%$, $81.342 \pm 3.224\%$) respectively compared to untreated diabetic rat group ($59.645 \pm 1.250\%$). Also percentage of dead sperm significantly ($p<0.05$) increased ($40.355 \pm 1.250\%$) in untreated diabetic rat group compared to control rat group ($14.911 \pm 0.758 \%$). In addition diabetic rat group treated with almond oil, Vitamin-E, L-carnitine, and (vitamin-E+L- carnitine) showed significant ($p<0.05$) decrease in the percentage of dead sperm ($24.800 \pm 0.786\%$,

$25.602 \pm 0.675\%$, $27.668 \pm 0.794 \%$, $18.658 \pm 3.224\%$) (Table 4.1; Figure 4.2 and 4.3).

Sperm motility

Percentage of motile sperm significantly ($p<0.05$) decreased in untreated diabetic rat group ($57.187 \pm 2.436\%$) compared to the control rat group ($84.761 \pm 0.982\%$).

When diabetic rat group supplemented with almond oil, vitamin-E, L-carnitine, and (vitamin-E+L-carnitine) showed significant ($p<0.05$) increase percentage of motile sperm ($74.000 \pm 1.536\%$, $73.586 \pm 2.025\%$, $71.118 \pm 1.860\%$, $79.588 \pm 2.140\%$) respectively compared to untreated diabetic rat group ($57.187 \pm 2.436\%$). Also, the percentage of immotile Sperm significantly ($p<0.05$) increased ($42.815 \pm 2.436\%$) in untreated diabetic rat group compared to the control rat group ($15.238 \pm 0.982\%$). while treatment with almond oil, vitamin-E, L-carnitine, and (vitamin-E+L-carnitine) showed significant ($p<0.05$) decrease in the percentage of immotile sperm ($26.000 \pm 1.536\%$, $26.414 \pm 2.025\%$, $28.882 \pm 1.860\%$, $20.412 \pm 2.140\%$) respectively compared to untreated diabetic rat group ($42.813 \pm 2.436\%$) (Table 4.2, Figure 4.4 and 4.5).

Sperm morphology

Percentage of the typical morphology of sperm significantly ($p<0.05$) decreased in the untreated diabetic rats group ($84.000 \pm 0.707\%$) compared to the control rat group ($94.500 \pm 0.619\%$), but diabetic rats treated with vitamin-E, (L-carnitine + vitamin-E), showed significant ($p<0.05$) elevation in percentage of normal Sperm morphology ($93.200 \pm 0.583 \%$, $94.800 \pm 0.800\%$) respectively when compared to untreated diabetic rat group ($84.000 \pm 0.707\%$). While L-carnitine and almond oil treated rat showed no significant change ($p<0.05$) in

percentage of normal morphology of Sperm ($86.000 \pm 1.449\%$, $85.200 \pm 1.392\%$) when compared to untreated diabetic rat group ($84.000 \pm 0.707\%$). The abnormal sperm morphology percentage elevated significantly ($p<0.05$) in untreated diabetes rats ($16.00 \pm 0.707\%$) in comparison with control rat group ($5.500 \pm 0.619\%$). Treatment of diabetic rat group with vitamin-E, (L-carnitine+Vitamin-E), causes significant ($p<0.05$) reduction of the total abnormal sperms ($6.800 \pm 0.583\%$, $5.200 \pm 0.800\%$) respectively when compared to treated diabetic rat group ($16.00 \pm 0.707\%$), but there is no significant ($p<0.05$) change in percentage of abnormal sperms in the L-carnitine and almond oil treated rat group ($14.000 \pm 1.449\%$, $14.800 \pm 1.392\%$) respectively compared to untreated diabetic rat ($16.00 \pm 0.707\%$) (Table 4. 2, Figure 4-6 and 4. 7).

Sperm motility and sperm morphology

The current study showed that Sperm motility percentage and sperm normal morphology rate were significantly reduced in diabetic rat group when compared to the control rat group. The present results are in agreement with the results obtained by (Fernandes et al., 2011; Abbasi et al., 2013).

Sexual dysfunction in diabetes animals may result from diabetes-induced alterations of the neuro-endocrine tract axis causes damage of the epididymis, with a negative impact on sperm (Seethalakshmi, 1987). Moreover, oxidative stress in testis of mice is associated with DNA damage and produces a higher frequency of abnormal sperm shapes; this has consequence significant effect on male fertility (Rajesh et al., 2002). In the present study the supplementation of diabetic rats with vitamin-E, have been improved sperm motility when compared to the control rat group. These results are concurrences with

other finding (Pena et al., 2003). Also these results may be explained by Verma and Kanwar (1999), concluding that vitamin-E supports sperm antioxidant system to improve sperm motility since the application of antioxidants including vitamin-E has been shown to enhance sperm viability. Vitamin-E supplementation has been shown to decrease sperm abnormality in diabetic rats this result agreed with Jamaludin (2012) who, reported that administration of vitamin-E to diabetic rats attenuated the spermatogenesis disruption in the testis induced by uncontrolled hyperglycemia in male rats. The dietary supplementation with vitamin-E has influenced directly or indirectly the spermatogenesis by non-antioxidant effects, during the second half of spermiogenesis, replacement of somatic histones by sequential expressions of spermatid nuclear transition protein (TP)-1, TP-2, and protamine results in the condensation of spermatid nuclei and initiates morphogenesis of the sperm head (Grimes et al., 1990). The abnormal sperm chromatin arrangement and impaired DNA wrapping have related to reduced expression of spermatid nuclear proteins.

In the current study L-carnitine supplementation to diabetic rat group improved sperm motility. This result agreed with previous study (Kang et al., 2011; Abo-Ghanema et al., 2012) reported that L-carnitine improves reproductive function via increasing sperm parameters, testicular antioxidant enzyme and testosterone hormone levels, L-carnitine is significantly correlated with sperm count, motility and vitality. While the result of the study sperm abnormal morphology percentage insignificantly changed this result agreed with that documented by (Khademi et al., 2012) in contrast L- carnitine supplementation has improved sperm morphology which documented by (Dehghani et al., 2013). Other studies have

suggested that L-carnitine has improved sperm motility and chromatin quality via antioxidant properties and the enhanced glucose uptake by sperm (Aliabadi et al., 2012). While the treatment with L-carnitine unable to improve total sperm abnormality, this may be due to dose-dependent, route.... of administration or may be due to its alone.

Wang et al. (2010), showed that L-carnitine can increase ejaculatory sperm motility of men with asthenozoospermia. In another study, Shi et al. (2010) concluded that testicular sperm motility improved after

exposure to L-carnitine in vitro. Thus May be the due action of beta oxidation of fatty acid (Jeulin et al., 1996) because fatty acid metabolism occurs in the mitochondria of sperm middle-piece. It has demonstrated that L-carnitine regulates the amount of acetyl coenzyme A, which is essential for tricarboxylic acid cycle and energy production. Therefore, the increased motility of sperm by L-carnitine in this study might be due to the effects of L-carnitine on oxidative phosphorylation and energy production.

Table.1 Experimental design

| Groups | No. of Rats | Dose/volume | Duration |
|----------------------|-------------|--|----------|
| control | 6 | tap water | 5 weeks |
| DM control | 4 | tap water | 5 weeks |
| DM + vitamin E | 5 | 2000 IU/kg diet/day | 5 weeks |
| DM + L-carini | 5 | 5 gm/kg diet/day | 5 weeks |
| DM + vit E+ L-carini | 5 | 2000 IU/kg diet/day + 5 gm/kg diet/day | 5 weeks |
| DM + Almond oil | 5 | 1ml/kg b.w. /day in diet | 5 weeks |

Table.2 Effect of almond oil, vitamin-E and L-carnitine on sperm count and sperm viability in diabetic male rats

| parameters | Sperm count | Sperm viability | |
|----------------------|-----------------------|----------------------|----------------------|
| | | Live%* | Dead%* |
| Standard control | 36 ± 2.768^c | 85.088 ± 0.758^c | 14.911 ± 0.758^a |
| Diabetic | 19.25 ± 1.250^a | 59.645 ± 1.250^a | 40.355 ± 1.250^c |
| DM + Almond oil | 31 ± 3.363^{bc} | 75.200 ± 0.786^b | 24.800 ± 0.786^b |
| DM +Vitamin- E | 25 ± 1.303^{ab} | 74.398 ± 0.675^b | 25.602 ± 0.675^b |
| DM +L-Carnitine | 31.2 ± 4.042^{bc} | 72.332 ± 0.794^b | 27.668 ± 0.794^b |
| DM +L- Carni +Vit- E | 32.2 ± 0.860^{bc} | 81.342 ± 3.224^c | 18.658 ± 3.224^a |

Values expressed as mean \pm S.E. The different letters indicate significant differences * = $p<0.05$, ** = $p<0.01$

Table.3 Effect of almond oil, vitamin-E and L-carnitine on sperm motility and sperm morphology in diabetic male rats

| Parameters Groups | Motile Sperm %* | Immotile Sperm %* | Normal morphology% * | Abnormal morphology %* |
|-----------------------|----------------------|----------------------|----------------------|------------------------|
| Standard control rats | 84.762 ± 0.982 c | 15.238 ± 0.982 a | 94.500 ± 0.619 b | 5.500 ± 0.619 a |
| Diabetic | 57.187 ± 2.436 a | 42.813 ± 2.436 c | 84.000 ± 0.707 a | 16.00 ± 0.707 b |
| DM+Almond oil | 74.000 ± 1.536 b | 26.000 ± 1.536 b | 85.200 ± 1.392 a | 14.800 ± 1.392 b |
| DM+Vitamin-E | 73.586 ± 2.025 b | 26.414 ± 2.025 b | 93.200 ± 0.583 b | 6.800 ± 0.583 a |
| DM+L-carni. | 71.118 ± 1.860 b | 28.882 ± 1.860 b | 86.000 ± 1.449 a | 14.000 ± 1.449 b |
| DM+L-carni+Vit- E | 79.588 ± 2.140 c | 20.412 ± 2.140 a | 94.800 ± 0.800 b | 5.200 ± 0.800 a |

Values expressed as mean \pm S.E. The differences letters mean significant differences * = $p<0.05$, ** = $p<0.01$

Figure.1 Effect of almond oil, vitamin-E and L-carnitine on sperm count in diabetic male rats

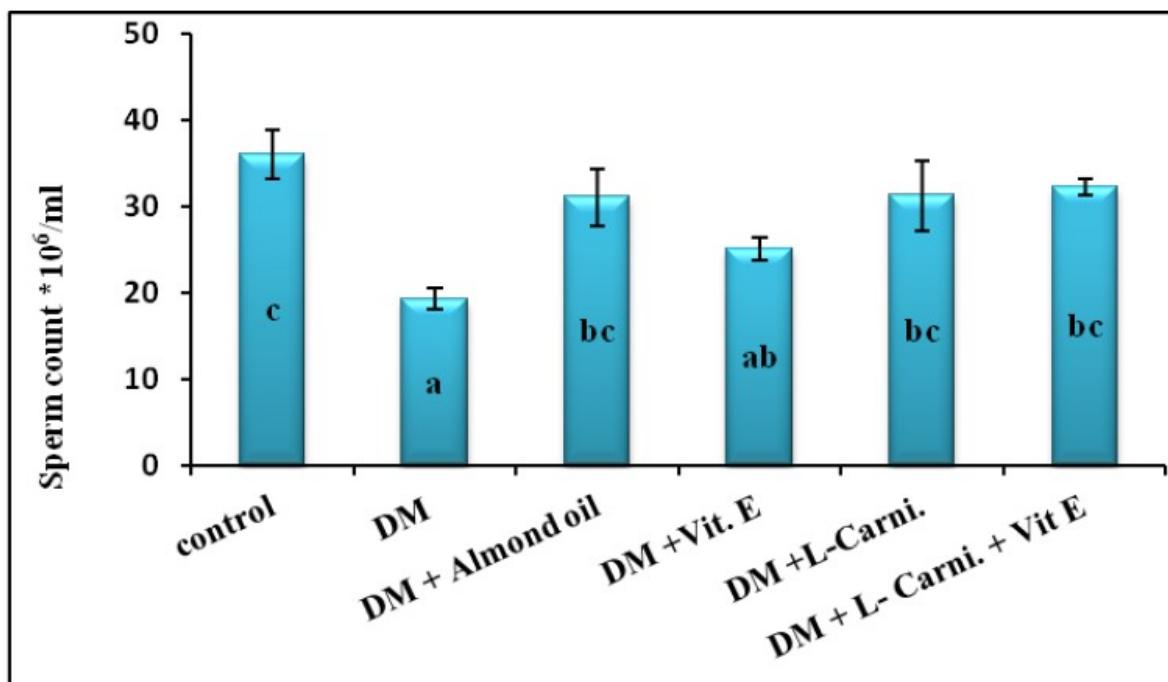


Figure.2 Effect of almond oil, vitamin-E and L-carnitine on live sperm in diabetic male rats

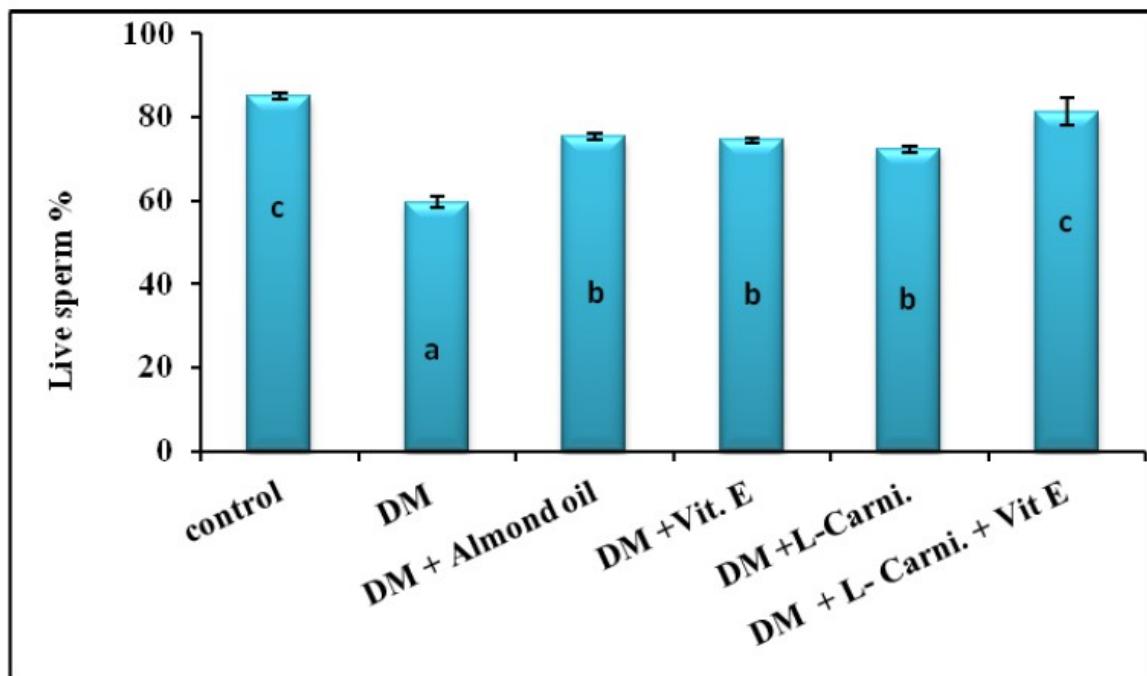


Figure.3 Effect of almond oil, vitamin-E and L- carnitine on dead sperm in diabetic male rats

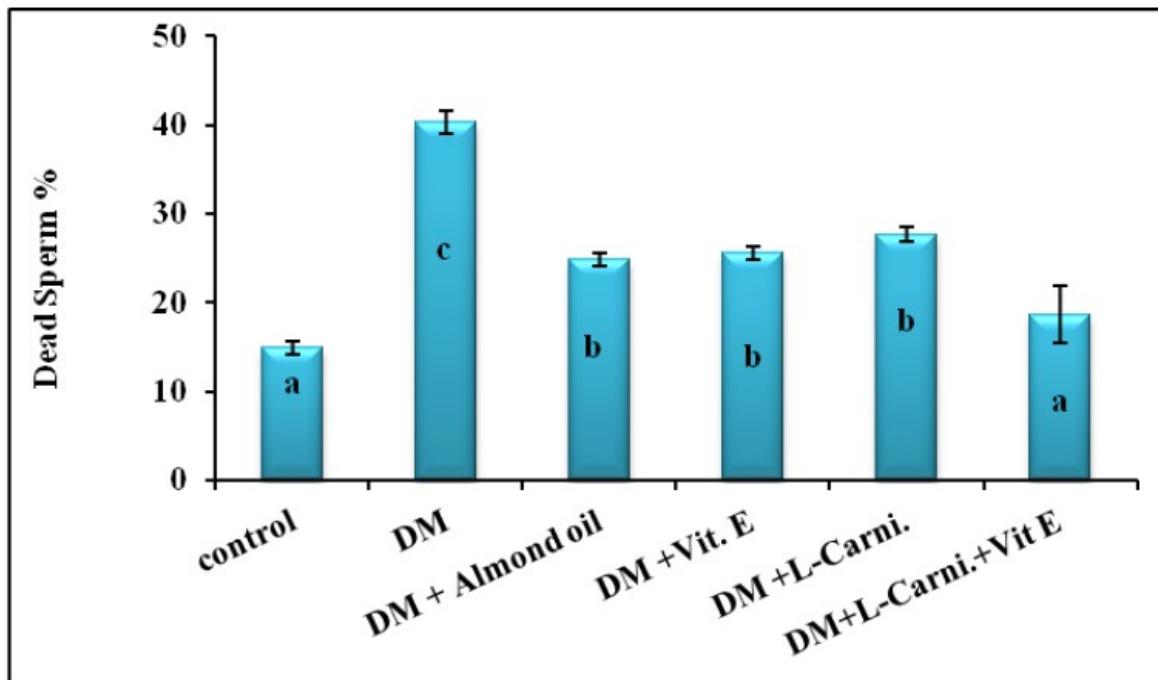


Figure.4 Effect of almond oil, Vitamin-E, and L- carnitine on motile sperm in diabetic male rats

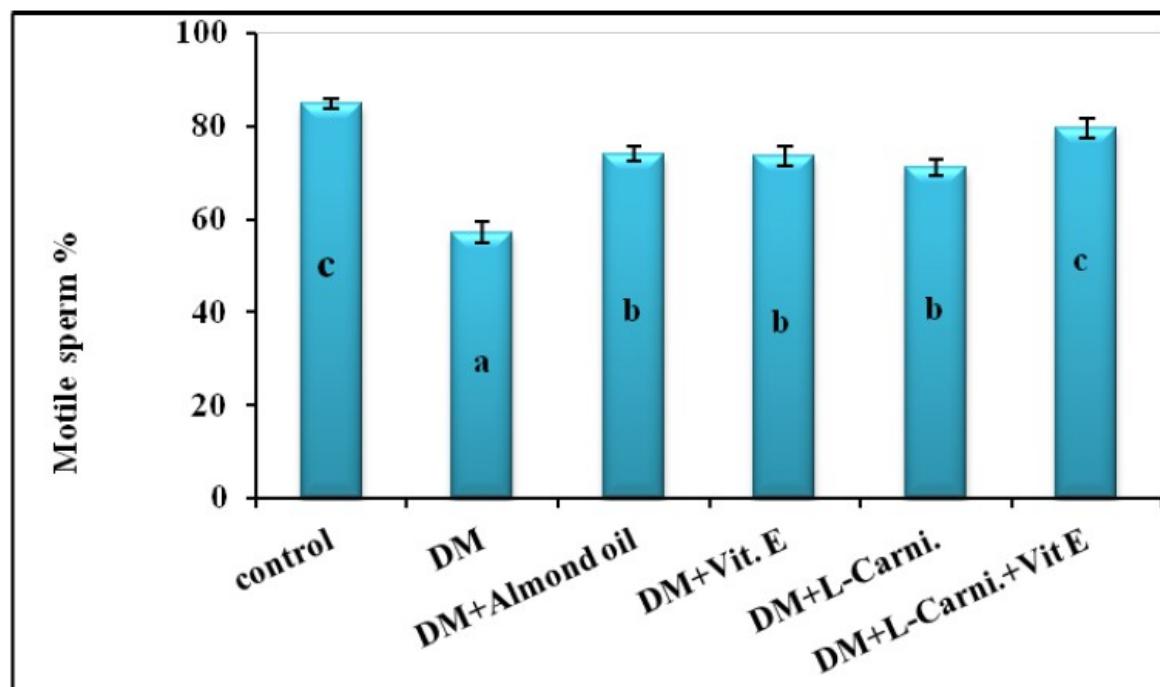


Figure.5 Effect of almond oil, vitamin-E and L-carnitine on immotile sperm in diabetic male rats

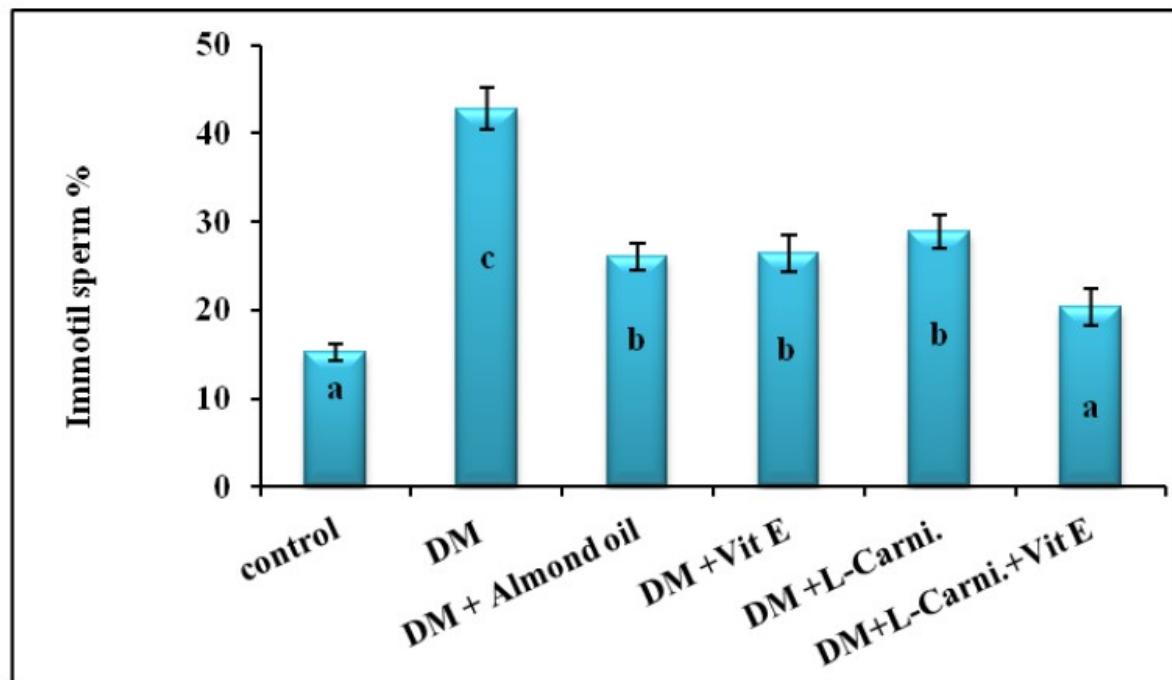


Figure.6 Effect of almond oil, vitamin-E and L-carnitine on normal sperm morphology in diabetic male rats

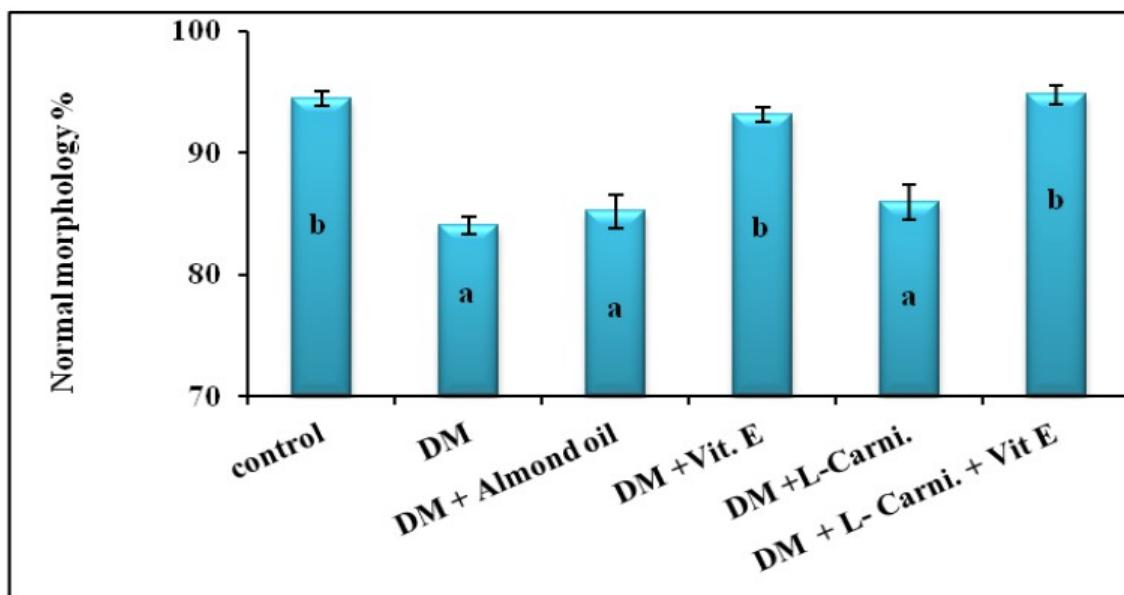
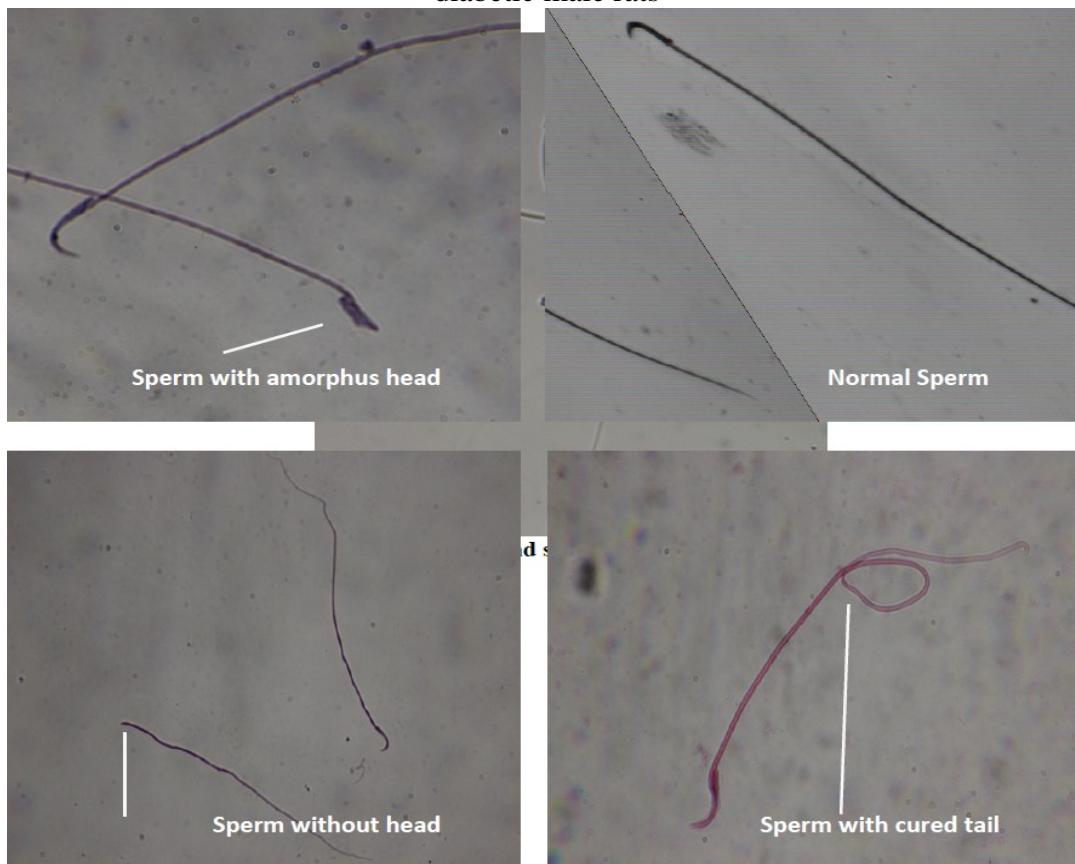


Figure.7 Effect of almond oil, vitamin-E and L-carnitine on abnormal sperm morphology in diabetic male rats



Administration of (vitamin-E+L- carnitine) to diabetic rats improves sperm abnormality. However, there's no more data about the use of Vitamin-E and L-carnitine in the form of combination, which may be due to their synergistic effects. *Prunus amygdalus* mainly increases the sperm motility and sperm contents in the epididymis and vas deferens without producing any spermatotoxic effects (Qureshi, 1989). These activities may be related to the presence of flavonoids and other phenolic compounds in almond oil such as Catechin, Gallo Catechin, Gallic acid, P-Covmaric acid, chlorogenic acid, Quercetin, Kaempferol isomers Epicatechin, and Tocopherol (Mazinani et al., 2012). The antioxidant function of α - tocopherol documented, but it is now evident that depending on the dose levels, α -tocopherol may exhibit and prevents free radical-induced injury by blocking the free radical chain reaction. Oil composition of, almonds are monounsaturated oleic acid and omega-9 fatty acid, linoleic acid a polyunsaturated omega six essential fatty acid and palmitic acid a saturated fatty acid (Berry et al., 1992).

Polyunsaturated fatty acids (PUFA) are the precursors of prostaglandins and leukotrienes, important factors in both sperm motility and inflammatory processes. Prostaglandin E and 19-hydroxy-prostaglandin E have been shown a relation to sperm motility. Forward sperm motility is seen when the concentrations of these substances lie within a relatively limited range of normality (Isidori et al., 1980).

This result indicates that sperm abnormal morphology percentage reduced in this study, may due to the protection of sperm DNA by the antioxidant effect of the almond oil. It may suggest that monounsaturated fatty acids stimulate enzyme activity and androgen secretion into the blood. Also

Anwar et al. (2013) reported that diabetic rats received almond oil, showed the lower percent of DNA damage. Lenzi et al. (1996), concluded that phospholipids and PUFA found in sperm membranes that participate in sperm cell structure and have a highly specialized scavenger system that defends the sperm membrane against lipoperoxidation.

The combination of vitamin-E with L-carnitine improve sperm count, sperm viability percent, sperm motility percent, normal morphology percent Almond oil shows more potency in protecting against alloxan-induced diabetes mellitus in rats and sperm damages than Vitamin - E and at the studied doses effects of almond oil.

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