

Changes in the mitochondrial electron transport chain (ETC) enzyme activities, ultra structure and motility of the sperms of lysergic acid (LSD) users

[Liserjik asit (LSD) kullananların spermlerinde mitokondriyel elektron taşınım zinciri (ETC) enzim aktiviteleri, ultrayapı ve hareketlilikte gözlenen değişiklikler]

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ABSTRACT

Lysergic acid diethylamide (LSD) is considered as the primary drug of hallucinogen class. This is the first study to report on the effect of LSD abuse on ultra structure and electron transport chain enzyme activities of sperms of LSD users which aimed to investigate the relationship between the sperm motility and mitochondrial electron transport chain enzymes activities in LSD users. The results showed that semen samples of LSD users (n = 15) have significantly lower sperm motility and lower activities of complexes I, II, III, IV and citrate synthase as compared with those of controls. Moreover, a direct and positive correlation was found in the whole population studied between spermatozoa motility and all the mitochondrial electron transport chain enzymes activities assayed (complexes I, II, III, and IV). In addition, the ultra structure study of sperms revealed that LSD mainly induces degenerated mitochondrial sheath with large cytoplasmic droplets. In conclusion, LSD impairs sperm motility through its direct toxic effect on sperm mitochondria leading to decreased mitochondrial energy production.

Key Words: Lysergic acid diethylamide, mitochondrial electron transport chain, sperm mitochondrial ultra structure.

ÖZET

Liserjik asit dietilamid (LSD), halusinojenik grubun primer ilacı kabul edilmektedir. Bu çalışma, LSD süistimalinin LSD kullanıcılarının spermlerinde ultrayapı ve mitokondriyel elektron taşınım zinciri enzim aktiviteleri üzerindeki etkisini rapor eden ilk çalışma olup LSD kullananlarda sperm hareketliliği ve mitokondriyel elektron taşınım zinciri enzim aktiviteleri arasındaki ilişkiyi incelemeyi amaçladı. Sonuçlar, LSD kullanıcılarının meni örneklerinde (n= 15) sperm hareketliliğinin ve I, II, III, IV kompleksleri ile sitrat sentaz enzim aktivitesinin azaldığını gösterdi. Bunun da ötesinde çalışılan grupta spermatozoa hareketliliği ile tayin edilen mitokondriyel elektron taşınım zinciri enzim aktiviteleri (Kompleks I, II, III ve IV) arasında doğrudan ve pozitif bir ilişki bulundu. Ayrıca, spermlerin ultra yapı çalışması, LSD'nin büyük stoplazmik damlacıklı mitokondriyel tabakanın dejenerasyonunu indüklediğini gösterdi. LSD'nin sperm mitokondrisi üzerinde doğrudan toksik etki göstererek mitokondriyel enerji üretimini azalttığı fikrine varıldı.

Anahtar Kelimeler: Liserjik asit dietilamid, mitokondriyel electron taşınım zinciri, sperm mitokondriyel ultra yapısı

INTRODUCTION

Lysergic acid diethylamide (LSD) is the primary drug of the hallucinogen class which is synthesized from ergotamine tartrate (derived from an ergot fungus on rye), or from lysergic acid amide (a chemical found in morning glory seeds or Hawaiian Baby Woodrose)⁽¹⁾. LSD is the most potent psychoactive drug with doses as small as 1.0 to 1.5 $\mu\text{g}/\text{kg}$ producing psychoactive effects. The psychoactive properties of the hallucinogen LSD have frequently been attributed to high affinity interactions with serotonin 5-HT₂ receptors in brain⁽²⁾. The hallucinatory effect of LSD can alter the sensory perception, states of consciousness and thought processes. All of these effects have contributed to the increase in LSD use⁽³⁾.

The continuous use of hallucinogenic drugs and of LSD in particular, raises concern regarding their short and long-term adverse consequences. The epidemiological studies suggest that LSD abuse is in increase among the young people whereas the use of the other substances continues to fall⁽⁴⁾. Although, LSD is not considered as an addictive drug since it does not produce compulsive drug-seeking behavior and most users of LSD voluntarily decrease or stop its use over time, LSD does produce a physiologic tolerance requiring subsequent increased doses for the same effect. So LSD must be considered as important as other drugs of abuse in terms of total damage caused by it to human population^(5,6). Deaths from LSD overdose apparently have not been previously confirmed toxicologically, however, high liver tissue level of lysergic acid diethylamide (LSD) was measured in many cases in which autopsy showed no obvious causes of death⁽⁷⁾.

Most of studies concerning LSD toxicity focus on the association of LSD use with neurotoxicity⁽³⁾. In vitro and in vivo studies have also indicated that LSD might cause structural changes in the chromosomes⁽⁸⁾, dynamically influences the expression of a small collection of genes within the mammalian prefrontal cortex⁽⁹⁾, genetic mutations, disturbances of embryonic development, and malignant degeneration of cells⁽¹⁰⁾. However, the toxic effects of LSD on male germ tissue are still being debated and no conclusive results have been obtained. It was hypothesized that LSD induces infertility in experimental animals as it was shown that LSD induces meiotic chromosomal abnormalities in mice that could have influence on fertility⁽⁸⁾. Although very few and limited data concerning the toxic influence of LSD on human germ cells are available, it was suggested that LSD carries the risk of inducing permanent or temporary infertility in human males. However, the mechanism is still unknown. Since LSD appears in the circulation and presumably has free access to germ cells, LSD could induce chromosome break points and sperm cell abnormalities

in human sperms⁽¹¹⁾.

Several recent reports have considered the possibility that mitochondrial dysfunction could be implicated as a factor in infertility and it is suggested that mitochondria play a key role in the energy maintenance of spermatozoa motility, the one of the major determinants of male fertility⁽¹²⁾. The common final pathway in the mitochondrial energy metabolism is the electron transfer chain, composed of two mobile carriers (coenzyme Q and cytochrome C) and four multimeric enzymatic complexes (complexes I, II, III, and IV), all embedded in the inner mitochondrial membranes⁽¹³⁾. Biochemical defined alterations of these respiratory chain activities are usually associated with all kinds of mitochondrial disorders⁽¹⁴⁾. LSD toxic effect on human germ cells remains an open question and no conclusive data have been obtained in this field. The aim of the present study is to confirm that LSD might exert its toxic effects upon sperm cells by affecting sperm mitochondrial function.

MATERIALS AND METHODS

The present study was carried out on 30 males of university students who were non-smokers and had similar ages and socioeconomic lifestyles. They were divided equally into 2 groups, as control and LSD users groups. The control group consisted of fifteen healthy students, ages ranged between 17-20 years with average of 20.9 ± 1.3 . The fifteen LSD male users gave a history of LSD intake for a period ranged between 1.5 to 3.5 years with mean duration of 2.8 ± 0.3 years. Their age ranged between 18 and 21 years old with mean age, 20.2 ± 2.1 years. They used LSD by placing drops of solution onto a piece of absorbent paper such as blotter paper with adhesive substance on its inner surface. It is divided into small decorated squares, with each square representing 1 dose, then they made small scratches on the inner side of the arm and put the paper over it, its street name is "stamp". According to US Drug Enforcement Administration reports, LSD dose in each stamp ranged between 20-80 μg and its effect could last from 8 to 10 hours⁽¹⁵⁾. The subjects of this study attended to Tanta Psychiatry Hospital (Gharbia government, Egypt) for treatment. Their clinical examination revealed that, 8 of them had panic reactions, and the remaining 7 subjects suffered from prolonged schizoaffective psychoses, however all of them had post-hallucinogen perceptual disorders. All the participants of this study were informed about the purpose of the work. An informed consent was obtained from each one before sample collection.

Ejaculates were obtained from each of the subjects of this study by masturbation into sterile plastic containers after 3 days of sexual abstinence. They were free from genital tract infections and did not receive any antibiotic therapy. Each ejaculate was divided into 3 parts,

one for the assessment of sperm motility, the second for ultra-structural microscopic study of the sperms and the third for the measurements of sperm mitochondrial ETC and citrate synthase (a reliable enzymatic marker of mitochondrial volume) enzymatic activities.

Sperm motility assessment:

Seminograms were performed according to WHO recommendations⁽¹⁶⁾. The concentration of spermatozoa was determined using the haemocytometer method. In order to measure the motility of the spermatozoa, the microscopic field was scanned and the motility of each spermatozoon was encountered. It was graded as a, b, c and d, according to whether it shows: (a) A rapid progressive motility, moving swiftly in a straight line; (b) A slow or sluggish progressive motility, less linear in their progression; (c) A non- progressive motility or (d) the immotility. The sum of classes (a) and (b) are taken as the progressive spermatozoa. Spermatozoa vertical progression was estimated by the introduction of a capillary tube filled of a 4.8% glucose isotonic solution, vertically on a semen sample and allowing the entrance of the spermatozoa for 30 min. After that period of time, the distance in millimeters that the spermatozoa were able to progress along the capillary tube was estimated. Spermatozoa vitality was determined by the eosin-nigrosin technique and the morphology was assayed by microscopy. The proportion of sperm cells with apparent head defects, mid piece malformations or tail abnormalities were estimated. According to WHO recommendations, asthenospermia was assigned to semen samples with less than 50% progressively motile spermatozoa. No patients with signs of flagella disturbance (very low motility but healthy vitality) was recorded.

Sample preparation for electron microscopic study:

The sperm suspension was centrifuged at 100 x g for 1 min to sediment the tissue fragments, and the supernatant was re-centrifuged at 1500 x g for 10 min at 4°C⁽¹⁷⁾. Sperm pellets were used in the extraction and fractionation.

Sperm extraction and fractionation:

Briefly, spermatozoa were suspended into a Tris-saline-protease inhibitor solution pH 7.5 (TNI=150 mM ,NaCl, 25 mM Tris-HCl, , 2 mM benzamidine, 1 µg/ml leupeptin, 1 µg/ml pepstatin, and 0.05% sodium azide) containing 0.1% Triton X-100 (TNI-Triton) at 4°C and then centrifuged at 1500 x g for 10 min; the sperm pellet was suspended into TNI-Triton solution containing 0.5 M NaCl and extracted 1 h at 4°C. The sperm suspension was homogenized with a glass-Teflon

Homogenizer, mixed with five volumes of a solution containing 43% Percoll (Amersham Pharmacia Biotech, Piscataway, NJ), 0.25 M sucrose, and 0.1% Triton X-100

in 50 mM Tris-HCl, pH 7.5, and centrifuged at 28 000 rpm for 35 min in a Beckman 60Ti rotor. The acrosomal matrices banded at the top of the gradient were collected and pelleted by centrifugation at 35 000 rpm for 60 min in a Beckman SW41 rotor⁽¹⁸⁾. Ultrathin sections were cut with LKB ultramicrotome and stained with uranyl acetate and lead citrate for detailed examination of sperms, which have been photographed with JEOL. JTEM.C.S.10 later.

Measurement of the mitochondrial electron transport chain (ETC) enzymatic activities in sperm samples:

Sample preparation:

Fresh semen (0.5-2 mL) was centrifuged at 600x g for 10 min, at room temperature. Seminal plasma was separated and the sperm pellet was washed twice with saline, and then once with phosphate-buffered saline. Pellet was frozen at -80°C for at least 15 min. After thawing, the pellet was suspended in 2 ml isolation buffer (0.25mol/l sucrose, 1 mmol/1EGTA, 10 mmol/1 HEPES, pH 7.4. and 0.5% bovine serum albumin) and centrifuged for 2 min at 500x g. The supernatant was discarded, the pellet was suspended in 3 ml isolation buffer and homogenized using a tight fitting homogenizer After centrifugation (10 min at 1500 x g), the supernatant was kept on ice and the pellet was rehomogenized and centrifuged as described above. The two supernatants were combined and centrifuged at 10,000 x g for 10 min. The resulting mitochondrial pellet was washed twice with the albumin-free isolation buffer and stored at -80°C until used. For the enzyme assays and the protein determinations, the pellet was suspended in 1 ml of water. The protein contents of mitochondrial suspension were determined by the Lowry method with bovine serum albumin as a standard⁽¹⁹⁾.

Electron transport chain complexes citrate synthase assay:

The activity of complex I (NADH :ubiquinone oxidoreductase) was determined according to the method described previously by Rustin, (1994)⁽²⁰⁾. Decylubiquinone was used as an electron acceptor at a final concentration of 75 mmol/l with rotenone (final concentration 5 mmol/l) for inhibition of complex I activity. The activity of complex II (succinate-ubiquinone oxidoreductase) was determined as previously described by Krahenbuhl et al., (1991)⁽²¹⁾ and complex III (ubiquinone -ferricytochrome c oxidoreductase) was determined according to the method of Krahenbuhl et al., (1994)⁽²²⁾. The activity of complex IV (ferrocytochrome c- oxygen oxidoreductase or cytochrome c oxidase) was determined according to the method of Wharton and Tzagaloff, (1967)⁽²³⁾. The activity of citrate synthase was determined by the method of Srere, (1969)⁽²⁴⁾.

Statistical analysis:

Results were expressed as means \pm SD. Comparison between groups were done using Student t- test. Data were considered significant at $p < 0.05$. Linear regression analysis was performed using simple regression analysis⁽²⁵⁾.

RESULTS

Table (1) demonstrated that LSD users have significant asthenozoospermia (range, 21–46%; $27.8\% \pm 7.1$) as compared with the controls (range, 55.0–75.0%; $57.8\% \pm 4.9$ %) ($p < 0.05$). Moreover, in LSD users significant decrease in the percentage of vital sperms (range, 49.0–65.0; 55.5 ± 4.8) were found as compared to the controls (range, 77.0–84.0; 78.8 ± 2.0) ($p < 0.05$). On the other hand, no significant differences were found between the controls and LSD users regarding to their spermatozoal concentration and the percentage of the abnormal spermatozoa ($p > 0.05$). The specific activities of the respiratory chain complexes I, II, III, IV and citrate synthase were determined in spermatozoa obtained from the controls and the LSD users. The results showed the significant decreases in the specific activities of the enzymes of LSD users as compared to those of controls ($p < 0.05$). (Table, 2). A positive correlation was detected between

the sperm motility and mitochondrial ETC enzyme activities (Fig 1,2,3, and 4).

Table 1. Seminal cellular parameters in the study subjects

	LSD group (n=15)	Control group (n=15)
Spermatozoa concentration	$93 \pm 11.2 \times 10^6$ /mL (78-100 $\times 10^6$)	$93 \pm 27.00 \times 10^6$ /mL (80-120 $\times 10^6$)
Asthenozoospermia (%)	27.8 ± 7.1 (21.0-46.0%)	57.8 ± 4.96 (55.0-75.0%)
Vital spermatozoa (%)	$55.5 \pm 4.8^*$ (49.0-65.0)	78.8 ± 2.07 (77.0-84.0)
Abnormal spermatozoa (%)	$32.66 \pm 4.5^*$ (35.0-49.0)	35 ± 7.4 (23.0-31.0)

Data are expressed as means \pm SD.

Ranges are shown in parentheses.

*Significantly different as compared to controls ($p < 0.05$).

Table 2. Mitochondrial enzyme activities in spermatozoa of the study subjects

Enzymatic activities (nmol/ min/ mg protein)	LSD group (n=15)	Control group (n=15)
Complex I	$538.5 \pm 51.6^*$ (480.0-612.0)	722 ± 40.8 (681.0-856.0)
Complex II	$32.2 \pm 4.6^*$ (26.0-41.0)	40.7 ± 4.5 (35.0-50.0)
Complex III	$620.0 \pm 41.5^*$ (580.0-745.0)	765.0 ± 20.2 (714.0-862.0)
Complex IV	$35.2 \pm 6.8^*$ (28.0-53.0)	44.6 ± 4.6 (39.0-53.0)
Citrate synthase	$211.3 \pm 33.9^*$ (170.0-260.0)	275.6 ± 42.5 (248.0-288.0)

Data are expressed as means \pm SD

*Significantly different as compared to control group ($p < 0.05$).

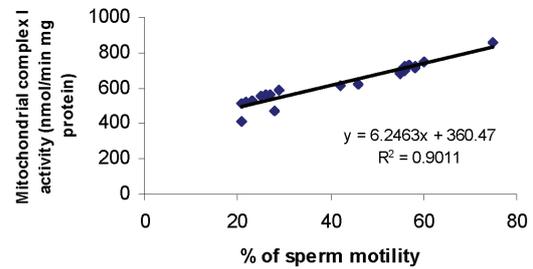


Fig. 1. Correlation between % of sperm motility and mitochondrial complex I activity among all the studied subjects.

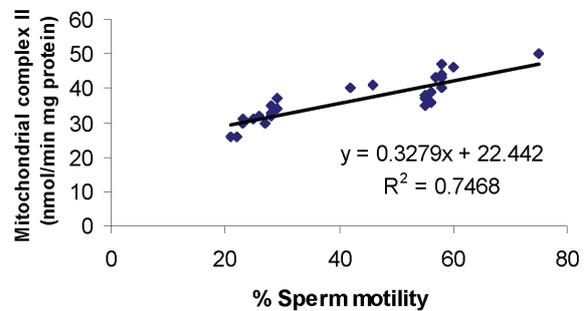


Fig. 2. Correlation between % of sperm motility and mitochondrial complex II activity among all the studied subjects.

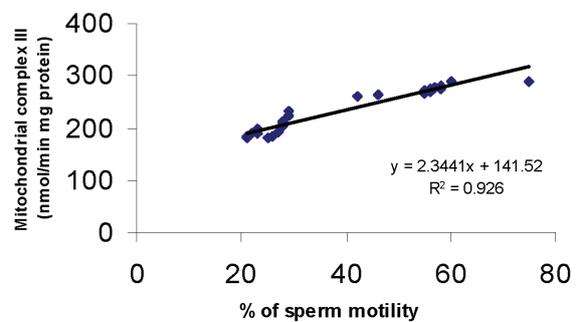


Fig. 3. Correlation between % of sperm motility and mitochondrial complex III activity among all the studied subjects.

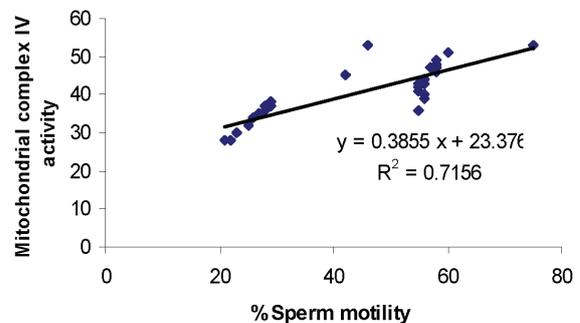


Fig. 4. Correlation between % of sperm motility and mitochondrial complex IV activity among all the studied subjects.

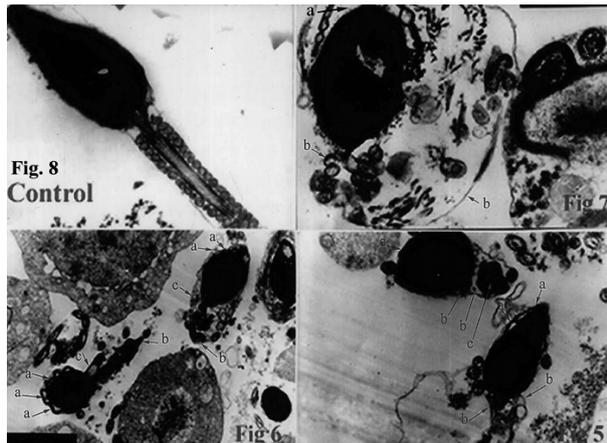


Fig. 5. An electronmicrograph of LSD users. The 2 malformed spermatozoa are seen. The head appears with irregular acrosome with peri-nuclear space (a). The mid-piece appears swollen with large cytoplasmic droplet with degeneration and disfigurement of mitochondrial sheath (b) Degeneration of axonomal structure (c). (EM X 8000)

Fig. 6. Malformed spermatozoa. The head shows irregular acrosome and perinuclear space (a) with breaking of the tail (b) and degenerated, disfigured mitochondrial sheath (c). (EM X5000)

Fig. 7. Severely altered acrosome with fuzzy appearance (a). A coiled tail with complete disorganization of both mitochondrial sheath and axonome (b). (EM X 14000).

Fig. 8. The normal control sperm.

Electron microscopic study of semen samples obtained from LSD users indicated malformed spermatozoa, with a head of irregular acrosome with peri-nuclear space (fig 5, 6) whereas some heads showed severely altered acrosome that has a fuzzy appearance (fig 7). The mid-piece appeared as swollen with large cytoplasmic droplets; the mitochondrial sheath showed degeneration and disfigurement (fig 5,6,7). The sperm tails showed coiling (fig 7), breaking (fig 6) and some of the cross sections of the tails indicated a degenerated axonomal structure (fig 5).

DISCUSSION

It has been previously reported that the factors affecting mitochondrial energy production may cause to sperm motility impairment. These factors include physiological conditions⁽²⁶⁾ or the well-known sensitivity of sperm to reactive oxygen species⁽²⁷⁾, toxicity of xenobiotics such as recreational drugs⁽²⁸⁾, or mtDNA mutations⁽²⁹⁾. Infertility, permanent or temporary, resulting from recreational drug use has become an important clinical problem. In recent years LSD appears as one of the mostly abused drugs particularly among the university students⁽³⁰⁾. It was reported that LSD is potentially toxic to gonads and low semen quality has been associated with its abuse⁽¹¹⁾.

The results of the semen analysis in the present study revealed that, there have been significant differences in the percentage of abnormal forms and sperm concentration in the semen of LSD users as compared to that of the control group. A significant asthenozoospermia was seen in LSD users as compared to controls. Since the subjects of LSD group were matched with the controls regarding the age, socioeconomic lifestyle and dietary habits, the significant asthenozoospermia found in LSD users mainly be attributed to the LSD toxic effects upon germ cells. In this study, one of the main findings was the presence of significantly decreased percentage of sperm vitality in LSD users as compared to control. Subjects with asthenozoospermia also exhibited the low sperm vi-

tility. This is in accordance with the findings of Eduardo et al., 2001⁽³¹⁾ who have recorded a direct and positive correlation between sperm vitality and motility. Ultra structure study of semen samples revealed that, LSD directly affected the sperm heads with the presence of acrosomes, with perinuclear space. The sperm tails showed breaking, coiling and degeneration of axonomal structure. However, the most obvious and prominent feature found was the degenerated disorganized mitochondrial sheath with large cytoplasmic droplet. Such observation suggested that LSD has a direct effect on mitochondrial volume of sperms⁽³²⁾.

The specific activities of the respiratory chain complexes; nuclear DNA encoded complex (II) and citrate synthase were found to be decreased in LSD users when compared to those of the controls. The direct and positive correlations with the motility and the mitochondrial encoded complexes, I, III, and IV in nuclear DNA-encoded complex II, and citrate synthase. These results provided a strong biochemical evidence to the finding that motility depends largely on the mitochondrial volume within the sperm midpiece. The reduced sperm motility observed in LSD users mainly be attributed to the sperm mitochondrial disorders. Folgero et al (1993)⁽³³⁾ have noted that the patients with primary mitochondrial disorders have shown reduced sperm motility.

In addition, PCR amplification of mitochondrial DNA (mtDNA) has shown a substantially higher incidence of a mtDNA deletion in patients with asthenozoospermia as compared with the unaffected individuals. mtDNA deletions are invariably associated with defects in mtDNA-encoded complexes I, III, and IV⁽²⁹⁾. However, we have observed a correlation with the motility and the complexes I, III and IV and nuclear DNA complex II which might not be explained entirely by the presence of different contents in the deleted mtDNA in LSD users.

The Linear relationship found in this study between mitochondrial specific enzyme activities and sperm motility in LSD users, clearly show a relationship between

mitochondrial energy production and cell motility.

The mechanism beyond which LSD induces sperm mitochondrial damage of its users is not clearly well established although it could be attributed to the sperm's well-known sensitivity to xenobiotics toxicity⁽²⁸⁾. It was assumed that LSD could have direct effects on sperm cells since it appears in the circulation and could gain access to the seminal fluid of its users.

LSD induced sperm mitochondrial damage of its users could also be attributed to the well-known LSD dopamine agonistic action. Most of the studies suggest that the dopamine agonists may affect the mitochondrial membrane potential⁽³⁴⁾. It has been postulated that dopamine metabolites and reactive oxygen species can directly alter protein function by the oxidative modification of mitochondrial proteins which may be the main targets for the oxidative damage.⁽³⁵⁾. Moreover, increased extracellular

concentration of dopamine may results in rapid and transient inhibition of mitochondrial function⁽³⁶⁾. It has been shown that, catecholamines may alter mitochondrial respiration⁽³⁷⁾. So it is strongly assumed that the toxic effect of LSD on sperm mitochondria occurs through its binding to the dopamine receptors in found in sperm cells leading to decreased mitochondrial energy production that directly leads to impairment of sperm motility.

In conclusion, the present study provides evidence that LSD have toxic effects on sperm mitochondria leading to a decreased mitochondrial energy production that directly leads to the impairment of sperm motility. The present study highly recommends further researches will be held on the binding of LSD to dopamine receptors in sperm cells through a mechanism in which LSD may induce toxicity in sperm cells.

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