



**Original Article:**

**Effect of Neem Oil on Sperm Mitochondrial Activity**

**Prashant Patil**, Department of Physiology, Laxminarayan Medical college and Research centre, Bhopal,

**Gaikwad RD**, Department of Physiology, Shantiram Medical College, Nandiyal

**Sawane MV**, Department of Physiology, Jawaharlal Nehru Medical College, Wardha,

**Waghmare VS**, Department of Physiology, People's College of Medical Sciences, Bhopal

**Address For Correspondence:**

**Dr. Prashant Patil,**

Prof & Head, Department of Physiology,  
Laxminarayan Medical college and Research centre,  
Kolar Road, Bhopal - 462042

**E-mail:** prashantpatil1411@gmail.com

**Citation:** Patil P, Gaikwad RD, Sawane MV, Waghmare VS. Effect of Neem Oil on Sperm Mitochondrial Activity. *Online J Health Allied Scs.* 2009;8(4):12

**URL:** <http://www.ojhas.org/issue32/2009-4-12.htm>

**Open Access Archives:** <http://cogprints.org/view/subjects/OJHAS.html> and <http://openmed.nic.in/view/subjects/ojhas.html>

Submitted: Oct 13, 2009; Accepted: Nov 19, 2009; Published: Apr 30, 2010

**Abstract:**

It is a known fact that neem oil has some effect on motility of sperm. Motility of sperm depends on mitochondrial activity present in mid-piece of sperm. In the present study, the mitochondrial activity of sperm was evaluated after treating semen with the different quantities of neem oil. The mitochondrial activity was also evaluated after subjecting the semen samples for different incubation periods keeping the quantity of semen as well as that of neem oil same. Tests were done on thirty normozoospermic semen samples with motile score more than 75%. It was found that as the quantity of neem oil increases, the mitochondrial activity decreases significantly ( $P < 0.001$ ). Similar results were found, when same quantity of neem oil was treated with same quantity of semen, but incubating for different time durations. The mitochondrial activity decreases significantly ( $P < 0.001$ ) from one minute to twenty minutes. So, it indicates that as the contact period between neem oil and semen increases the mitochondrial activity decreases significantly.

**Key Words:** Normozoospermia, Neem Oil, Sperm Mitochondrial Activity

**Introduction:**

Indigenously available neem oil in its natural form has spermicidal and anti-implantation effect.<sup>1</sup> Two derivatives of neem

oil – sodium nimbinat and sodium nimbidinate have been found to possess weak spermicidal action in vitro.<sup>2</sup> It was found that neem oil decreases sperm motility instantly.<sup>3</sup> In view of this, the present study was undertaken to find the effect of neem oil on sperm mitochondrial activity.

**Materials and Methods:**

The study was carried out in semen laboratory of Department of Physiology, Govt. Medical College, Nagpur. Semen sample of normozoospermic men were obtained after five days of abstinence. After proper liquefaction, at room temperature, sperm motility was checked. Thirty samples with more than 75% motile spermatozoa were taken for test, while the remaining samples were discarded. Neem oil was procured from Khadi Gramudhyog shop. Two types of tests were carried out. In first type of test, the quantity of neem oil was changed and incubation period was kept constant. [Table 1]

In second type of test, neem oil and semen quantities were kept constant and they were incubated for different time durations. [From one min. to twenty mins.] [Table 2]

After incubation (in first case for 5 min. and in second case from one min. to twenty mins), one drop of solution from respective test tube was drawn and SMAI (Sperm Mitochondrial Activity Index) was performed.

**Table 1: Incubation period kept constant (n=30)**

Test tube no	Quantity of semen (ml)	Quantity of neem oil (ml)	Incubation period (min)	SMAI score	P value
1	0.1	nil	5	73.32 ± 3.31	
2	0.1	0.1	5	68.52 ± 1.61	<0.001
3	0.1	0.2	5	44.18 ± 4.69	<0.001
4	0.1	0.3	5	32.07 ± 2.15	<0.001
5	0.1	0.4	5	20.17 ± 1.97	<0.001
6	0.1	0.5	5	10.98 ± 2.09	<0.001

**Table 2: Quantity of neem oil kept constant (n=30)**

Test tube no	Quantity of semen (ml)	Quantity of neem oil (ml)	Incubation period (min)	SMAI score	P value
1	0.1	0.1	1	62.23 ± 2.12	<0.001
2	0.1	0.1	5	51.13 ± 2.18	<0.001
3	0.1	0.1	10	41.88 ± 2.84	<0.001
4	0.1	0.1	15	21.92 ± 2.65	<0.001
5	0.1	0.1	20	13.32 ± 3.52	<0.001
6	0.1	Nil	20	74.15 ± 3.06	

#### SMAI Test<sup>4</sup>

##### Method

- One drop of semen and one drop of Nitroblue Tetrazolium (NBT) solution was put in a cavity slide.
- The cavity slide was covered with cover slip.
- It was kept in a Petri dish with moist cotton on the sides.
- It was incubated at 37° C for 30 minutes.
- It was mix well and a smear was prepared on a pre-cleaned labeled slide.
- It was allowed it to air dry.
- Then the slides were fixed in neutral formalin for 2 hours.
- After 2 hrs slides were wash with distilled water and allowed to dry.
- These slides were viewed using oil immersion lens.
- The extent of NBT precipitate of 100 spermatozoa as standard, substandard, low, residual and nil were counted.
- The percentage under each grade were multiplied with arbitrary value of 1, 0.7, 0.3, 0.1 and 0 for standard, substandard, low, residual and nil respectively.
- The total of all was the sperm mitochondrial activity index.

Such tests were carried out on 30 normozoospermic subjects in Dept. of Physiology, Govt Medical College, Nagpur. Statistical analysis was done by Student's t test.

##### Results:

In the first test, where incubation time was kept constant (5 min), it was seen that test tube with nil concentration of neem oil had  $73.32 \pm 3.31$  sperm mitochondrial activity index. As the concentration of neem oil was increased from 0.1 ml to 0.5 ml, the significant results were obtained for SMAI. [Table 1] In first test tube with 0.1 ml concentration of neem oil, SMAI score was  $68.52 \pm 1.61$ . It was highly significant when compared with test tube having no neem oil (test tube no. 1) ( $P < 0.001$ ). Similarly, as the concentration of neem oil was increased in other test tubes, it showed a significantly decreased SMAI score as compared to test tube no. 1 ( $P < 0.001$ ).

When concentration of neem oil was kept constant and incubation time was varied from 1 to 20 mins, it showed the following result. [Table 2] In test tube no. 6 (with no neem oil) the SMAI score was  $74.15 \pm 3.06$ . This shows that there was no obvious change in SMAI score after 20 mins of incubation. In test tube no. 1 to 5, SMAI score started decreasing significantly when incubation period was increased. When incubation period was 20 mins SMAI score was  $13.32 \pm 3.52$  (neem oil conc 0.1 ml). When these results were compared with test tube no. 6 they were found to be statistically highly significant ( $P < 0.001$ ). This shows that neem oil decreases the SMAI scores significantly with increasing incubation periods.

##### Discussion:

It is reported that neem oil has spermicidal effect and it reduces the motility of sperms.<sup>5-8</sup> Also, it increases the uterine contractions as reported by Mitra<sup>9</sup>, after application to Guinea pig uterus. Again, the suppression of ovulation by Azadiractin (a component of neem oil) is reported by Renbold & Sieber in locusts.<sup>10</sup>

The present study is mainly concerned with motility of sperm. Motility of sperm depends on flagella. It is reported that neem

oil has anti flagellar action.<sup>11</sup> Flagellar motility is due to mitochondrial activity in mid-piece of sperm. In the present study mitochondrial activity was measured after treating the semen with neem oil. It shows that with increase in quantity of neem oil, the mitochondria of sperm are affected more, thus affecting the sperm motility adversely. (Table 1).

When same quantities of semen and neem oil were incubated for different time durations it was found that the sperm mitochondrial activity decreased significantly as the incubation period increased. SMAI score was  $74.15 \pm 3.06$  in sample without neem oil, while it was  $13.32 \pm 3.52$  in sample with same quantity of neem oil when incubated at 37° C for 20 min (Table 4). This indicates that there is reduced motility of sperm after reacting with neem oil for 20 min. The neem oil is possibly acting upon the enzymes in mitochondria that are responsible for production of ATP. Thus the depletion of ATP is resulting in reduction of sperm motility.

##### Conclusions:

The above study is helpful in showing that neem oil is a potent agent having adverse action on mitochondria and thus reducing sperm motility. Spermicidal action of neem oil was also reported by K C Sinha et al.<sup>1,6,7,13</sup> Abundant availability of neem oil can make it a favourable agent of contraception in India. It can be an agent in non hormonal pre and post coital contraceptive. Further studies are needed.

##### References:

1. Sinha KC et al. Anti-implantation effect of neem oil. *Ind. J. M. Res.* 1984;80:708-710.
2. Sharma VN, Saxena KP. Spermicidal action of Sodium Nimbinat. *Ind Jour. M. Res* 1959;47:322-324.
3. Sinha KC, Tiwari RS. Neem oil as Vaginal Contraceptive. *Ind Jour. M. Res* Jan 1984;79:131-136.
4. Gopalkrishna et al. *Mol. Andrology* 1990;3:243-253.
5. Sharma VN et al. *Ind Jour. M. Res* 1959;47: 3.
6. Kasturi M, Ahmad RN et al. Mode of long term anti-fertility effect of intra-uterine neem treatment. *J Basic Clin Physiol Pharmacol.* 2002;13(4):311-28.
7. Sharma SK, Sairam M et al. Neem oil inhibits two cell embryo development and trophoblast attachment and proliferation in vitro. *Contraception.* Dec 1996;54(6):373-8.
8. Juneja SC, Williams RS et al. Immuncontraception undergoing promising trials. *J Assist Reprod Genet.* Aug 1996;13(7):576-85.
9. Mitra CR. In-Natural Pesticides from neem tree, proceedings of 1<sup>st</sup> International conference, Rottach Egren, Federal Republic of Germany, 16-18 June 1980. pg no. 97.
10. Rembold H, Seiber KP. In-Natural Pesticides from neem tree, proceedings of 1<sup>st</sup> International conference, Rottach Egren, Federal Republic of Germany, 16-18 June 1980. pg no. 75.
11. Chopra et al. *Indigenous Drugs of India*, 1982 Pg no. 962.
12. Newton P et al. A new family planning tool to slow population growth. *IDRC Rep.* Jan 1993;20(4):16-18.
13. Bardhan J, Riar SS et al. Neem oil – a fertility controlling agent in rhesus monkey. *IJPP.* Oct 1991;35(4):278-80.