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Neem Seed Extract: it's Effect(s) on Semen Quality and Lipid Peroxidation

Naiho A. O. * Department of Human Physiology, Faculty of Basic Medical Sciences, College of Health Sciences, Delta State University, Abraka, Delta State, Nigeria

Mukoro E. Department of Human Physiology, Faculty of Basic Medical Sciences, College of Health Sciences, Delta State University, Abraka, Delta State, Nigeria

Okonkwo B. C. Department of Human Physiology, Faculty of Basic Medical Sciences, College of Health Sciences, Delta State University, Abraka, Delta State, Nigeria

Odigie M. O. Department of Human Physiology, Faculty of Basic Medical Sciences, College of Health Sciences, Delta State University, Abraka, Delta State, Nigeria

Abstract: *Azadirachta indica*, locally called Dongo yaro is one ubiquitous native herb in Nigeria today. Though yet to be fully explored by orthodox and trado-medical practitioners, its positive and/or hazardous effect(s) remain(s) a "mysterious" to researchers, the academia, public and health care providers. In view of this, this study was instigated to investigate and chronicle (where found), the exact effect(s), that extracts from Dongo yaro (Neem Oil) will pose on the male reproductive gamete; specifically semen quality, as well as lipid peroxidation levels. To achieve this, semen from thirty (30) Normo-zoospermic males was obtained from Igbinedion University Teaching Hospital, Okada, Edo State. Specimens were then categorized under two groups (A and B) based on incubation periods per incrementally added quantities of semen and neem oil. With groups A and B respectively allotted 15 specimens (n = 15 each) for their respective subgroups (A1 through A15 and B1 through B15), test-tubes A1 through A3, and subgroup B1 (comprising of 3 specimen) were left untreated with Neem oil, thus acting as controls. photocolorimetric results show, upon comparison with other experimental groups (A4 through A3 and subgroups B2 through B5), that prolonged increase in the quantity of Dongo Yaro extract (Neem Oil) has a negative effect on male reproductive gametes, as it caused high reduction in semen quality while increasing lipid peroxidation levels as well.

Keywords: Neem; Neem plant; Neem seed; Neem oil; Neem seed oil; Azadirachta indica; Dongo yaro; Semen quality; Lipid Peroxidation.

1. Introduction

Botanically called ***"Azadirachta indica"***. The Neem plant, aka "Nimtree or Indian Liliac" [1] is a tree of the Eudicotylidonous class, belonging to the taxonomic family of Mahogany, Meliaceae in the plant (*Plantea*) kingdom. This "Omnipotent" plant typically grows in tropical and semi-tropical regions of the world. Native mainly to India, Nepal, Pakistan, Bangladesh, Iran and Sri Lanka, its fruits and seeds are the chief source of Neem oil [2].

Though shown to pose encephalopathic and ophthalmopathic effects in excess consumption [3]. Neem oil is thought of as a highly medicinal herbal extract that is suitable for man's use and consumption. In the Indian states of Andhra Pradesh and Karnataka, Neem seed oil is very popular for its use as anthelmintic, antifungal, antidiabetic, antibacterial, antiviral, contraceptive and as sedative agent [4]. In Unani Medicine, it is particularly prescribed for skin diseases like eczema, for improvement of Liver functions, blood detoxification or cleansing, healthy hair development and for a balanced blood sugar level [3]. Prior to the availability of written records, Neem extracts have been used by Mankind in the treatment of various ailments. The Neem tree is an incredible plant that has been declared the "Tree of the 21st Century" by the United Nations [5].

Recently, studies have shown that neem seed oil has some effects on semen quality [6] which in turn, is dependent on testicular mitochondrial activity [7]. Though investigation remains vague, several findings on the effects tend to slide towards the positive side. For instance, Piomboni et al., reported in 2012 [8] that Semen has an alkaline nature, and they do not reach full motility (hyper motility) until they reach the vagina where the alkaline pH is neutralized by acidic vaginal fluids. Ward and Coffey also reported in their 1991 findings that; sperm DNA was at least six folds more highly condensed When compared to mitotic chromosomes in somatic cells.

*Corresponding Author

ROS had been shown to pose both beneficial and/or detrimental effects on sperm functions depending on its nature and the concentration, as well as the location and length to its exposure [9].

This study ascertains and clarifies (where found), the actual effect(s) that neem seed oil will pose on semen quality. It also investigates the effect of the extract on cell membrane through lipid peroxidation activities.

2. Methodology

2.1. Resources and Sources

2.1.1. Human Semen

With assistance from the hospital authority, thirty (30) Normo-zoospermic males, who regularly attended check-up at Igbinedion University Teaching Hospital, Okada, Edo State, were recruited. Selection criterion was based on abstinence from sexual intercourse for at least, five days interval. Only semen of over 75% motility score was included for investigation. The decision to opt for 5 days minimum semen was informed by the account, that, prolonged avoidance of sexual intercourse will generate a 25-45% folds increase in ejaculated volume, mainly by increased prostate secretion; Thus, increasing motility as well [9]. Spermatozoids that fell below Kruger's criterion were excluded from the study.

2.1.2. Neem Seed Extract { Neem Oil }

Refer to procedure (below) for the extraction process.

2.2. Other Resources

Other used resources include; Universal sterile container, sterile test tubes, weighing balance, pipettes, Neuber's Counting Chamber (haemocytometer), binocular light microscope, spectrocolorimeter, Tris buffer solution (sterile water) and neem oil extract. All were readily available within the laboratory unit of the hospital wherein the tests were conducted.

2.2.1. Ethical Consent

Ethical consent/approval was sourced from the hospital management of the Igbinedion University Teaching Hospital, Okada, Edo State, as well as the Research and Ethics committee of the college of Health Science, Delta State University, Abraka, Delta State.

2.2.2. Procedure

Obtained specimens (semen) were categorized into two (2) main groups (A and B) based on variation of incubation periods per incremental addition of semen and neem oil. While group A received 15 collected semen (n = 15 specimens) for its subgroups (A1 through A15), group B (comprising of 5 subgroups of 3 specimens each as B1 through B5) equally have 15 semen test tubes. In either case, each subgroups of groups A and B (A1 through A15 and B1 through B15 respectively) were composed of 15 different specimen collections (n = 15) in each of its 15 test tubes; giving a total of 15 test-tube of A1 through A15 for group A, and 15 test-tube of 5 subgroups (3 specimen per group) for group B. In each group, test-tubes A1 through A3 and test-tubes B1 through B3 (first subgroups of group B) were respectively untreated with neem oil to act as their controls. Whereas, experimental test-tubes of group A (A4 through A15) received varying and steadily incremented quantities of neem oil at constant incubation periods (5 minutes), those of group B (3 specimens each in subgroups B4 through B15) were treated with fixed quantities of semen and neem oil at variable incubation periods (5, 10, 15, 20 and 25 minutes). In either test, the absorbance of each test tube was determined using photo-colorimeter. Obtained values were read appropriately and recorded.

2.3. Extraction of Neem oil

The process appeared tedious, spanning couple of days due to sun-drying. Firstly, Matured (ripe fruits) neem seeds of desired quantity are harvested from different location in Benin City, Edo state. Next, harvested seeds were repeatedly weighed and sun-dried; in each step, weighted values were noted as appropriate. At some point where weight became constant (indicative of adequate dryness), fruit's endocarp was cracked to obtain seed. The cold method (a mechanical press method) was used for extracting the oil from the seed kernel. Dried neem seed kernels were placed in a manually operated compressor to squeeze the kernels under pressure until the oil was pressed out and collected. Desired quantity (25ml) was then stored for this study, following removal of impurities from filtrate [10].

2.4. Counting the Sperm

Using the newly improved Neuber's counter; about 10µl of each semen sample were kept in the Counting Chamber for about 5 min. observations were then made with the aid of a light binocular microscope.

2.5. Obtaining the Motility Rate

Using the Tris buffer solution [11]. Samples from each subgroup were diluted with to 0.5 ml, and an aliquot of this solution was observed under the light binocular microscope. Mean motility was expressed in percentages.

2.6. The Morphology

Morphology of the spermatozoa was determined by using the original dilution for motility. Sperm cells were then categorized based on measuring system of the strict Kruger criterion. Findings were expressed as percentage of morphologically normal sperm.

2.7. Testing for Lipid Peroxidation

Using malondialdehyde (MDA) as a marker, we were able to estimate peroxidase activities of spermatozoa. Here, MDA production level was accessed by reacting with thiobarbituric acid (TBA). To achieve this, a 0.5ml of TBA reagent, standardized as 0.67 molar solution (0.67g/100ml) was added to 1ml of seminal plasma (supernatants) contained in a glass tube of 0.9ml water. Thereafter, the combination, which also had 0.5g NaOH and 100 ml glacial acetic acid, was heated for 1 hour in a boiling water bath (all samples ran as duplicates). After 10 minutes of cooling; supernatant absorbance of these were read (in nano- mole) on a photo-colorimeter (at 534 nm) following centrifugation. Note that The MDA Standards for Colorimetric Detection of 2.5, 5, 10, 20 nmole/ml MDA was used [12].

2.8. Statistical Analysis

Results were expressed as mean ± SD. The evaluation of data for statistical significance between control and experimental groups was done using ANOVA. Pearson Product Moment Correlation (PPMC) was used to establish relationship. A p-level of less than 0.05 was accepted as statistically significant.

3. Results

Firstly, we present the raw results in tabular form as obtained from the laboratory:

Table-1.1. Showing MDA concentration from photocolorimeter readings

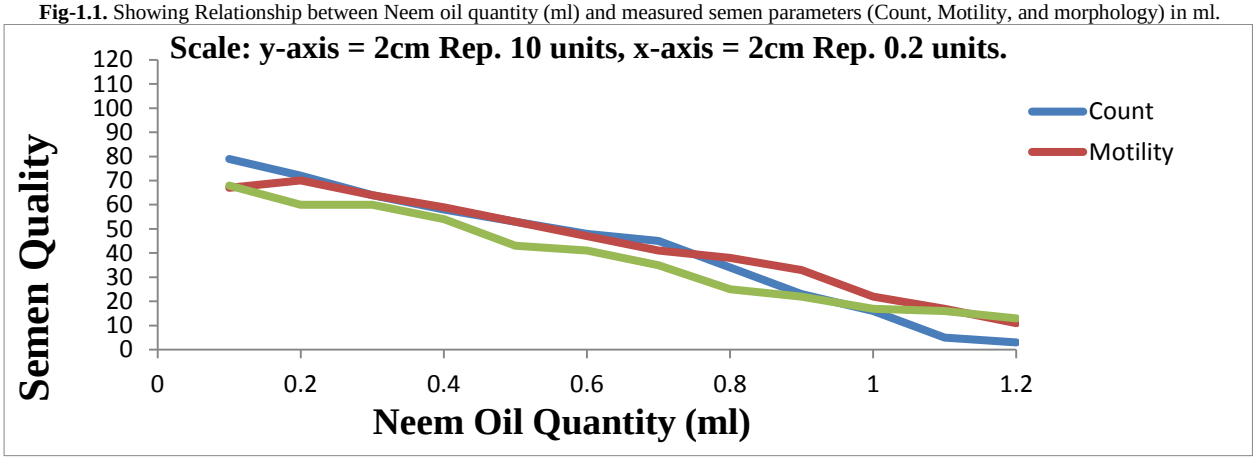
Test tube no.		Quantity of semen (ml)	Variable Quantity of neem seed extract (ml)	Constant Incubation period (min)	Photocolorimetric values of MDA in sample (nmole)
		1	Nil	5	
1A	CONTROL GROUP				0.55
2A		1	Nil	5	0.71
3A		1	Nil	5	0.86
4A		1	0.1	5	2.461
5A		1	0.2	5	3.29
6A		1	0.3	5	3.63
7A		1	0.4	5	4.02
8A		1	0.5	5	5.13
9A		1	0.6	5	4.93
10A		1	0.7	5	4.93
11A		1	0.8	5	4.77
12A		1	0.9	5	5.03
13A		1	1.0	5	7.46
14A		1	1.1	5	8.07
15A		1	1.2	5	8.13

Table 1.2. Showing Sperm Quality readings

Test tube no.		Sperm motility (%)	Sperm count x 106/ml	Morphology (%)
1A	CONTROL GROUP	89.7	80.0	69.7
2A		76.4	110.0	73.1
3A		83.9	107.0	73.8
4A		67.0	79.0	68.4
5A		69.8	72.0	60.3
6A		64.4	64.0	59.9
7A		59.1	58.0	54.1
8A		53.4	53.0	44.3
9A		47.5	48.0	41.3
10A		41.0	45.0	35.3
11A		35.7	34.0	25.4
12A		32.5	23.0	22.2
13A		22.3	16.0	16.9
14A		17.0	5.0	16.4
15A		10.5	3.0	13.4

ANOVA showed a statistical significance at $p < 0.05$, while Pearson returned inverse correlation with Neem seed extract and sperm count.

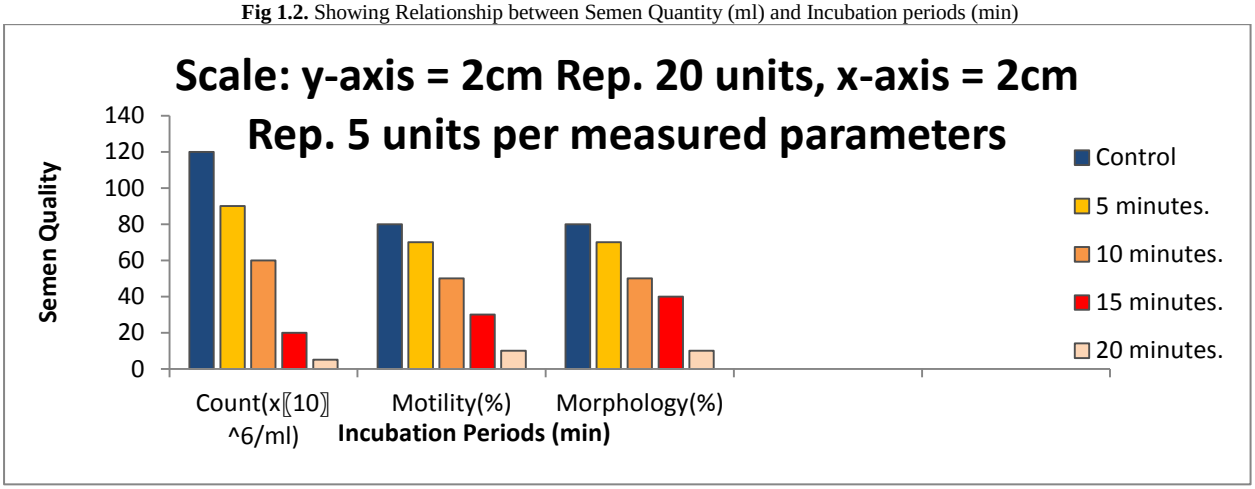
Fig-1.1. Showing Relationship between Neem oil quantity (ml) and measured semen parameters (Count, Motility, and morphology) in ml.



**Compared to control, while ANOVA proved significant at $p < 0.05$, Pearson returned $r = -0.973$, -0.989, and -0.993 for sperm count, motility, and morphology respectively.*

Figure 1.1 above shows a dose-dependent effect of Neem seed extract on sperm count. As observed, neem oil decreased the sperm count as extract concentration (dosage) increased. Fig. 1.1 also reports a dose dependent decrease in the percentage of normal sperm motility with an increased dosage of Neem oil, showing both as inversely correlated. Also, the figure shows a dose dependent effect of Neem seed extract on percentage of normal sperm morphology with an inverse correlation between Neem oil concentration and normal sperm morphology. The figure also shows that neem seed extract increased the MDA level with increased concentration, proving to be statistically Significant at $p < 0.05$, while positively correlating for Malonaldehyde level and neem oil concentration

Fig 1.2. Showing Relationship between Semen Quantity (ml) and Incubation periods (min)

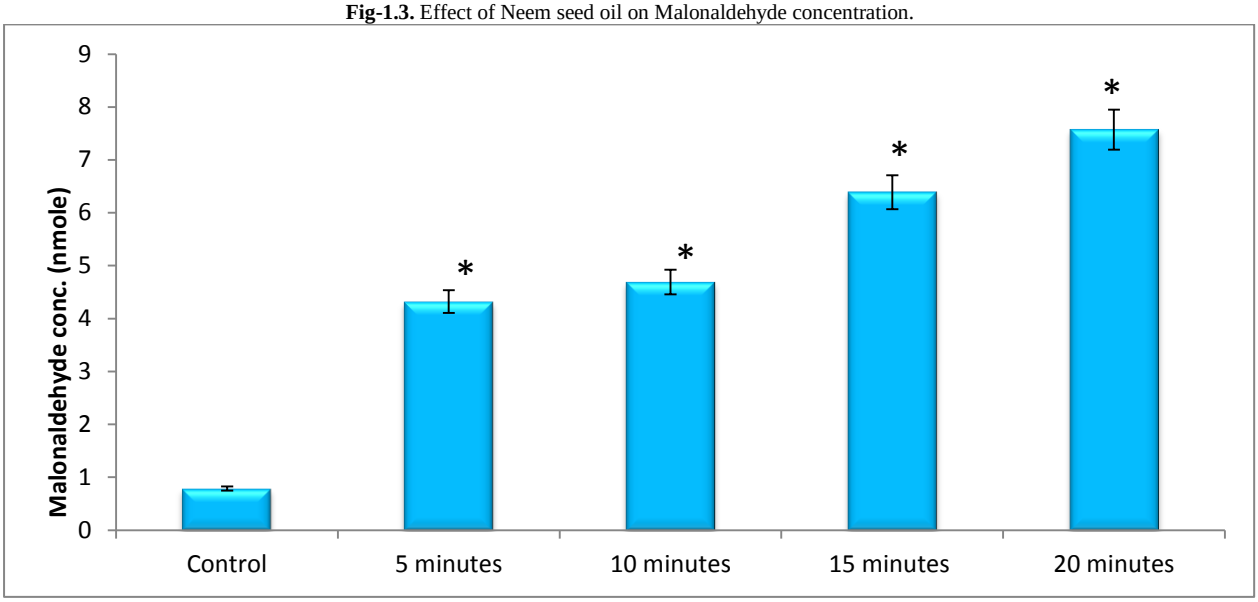


**p < 0.05 compared with control group (n=6)*

Figure 1.2 above shows duration dependent changes of Neem oil extract on indicators of semen quality (count, motility and morphology). As seen, sperm count decreased as exposure duration to neem oil increased. Statistically, this was significant at $p < 0.05$. However, upon exposed to neem oil for durations of 10, 15 and 20 minutes, an obvious decrease in sperm count was seen compared to those of the control, showing no statistical difference(s) for sperm cells treated with the same quantity of neem seed extract in periods less than 5 minutes. Fig 1.2 also shows that neem seed extract caused a duration dependent decrease in normal sperm motility. For morphological changes, the result was similar to those seen in motility. In both cases, the decrease in motility and morphology were significant at $p < 0.05$ for separate periods of exposure to neem seed extract as compared to the control.

Fig. 1.3 shows duration dependent effect of Neem seed extract on changes in MDA level. Here, what was observed is an increase in mean MDA level resulting from duration dependent effect of neem oil. This was statistically significant at $p < 0.05$ when compared to the MDA level of the control subgroup.

Fig-1.3. Effect of Neem seed oil on Malonaldehyde concentration.



**p < 0.05 compared with control group (n=6)*

4. Discussion

Overtime, Neem seed extract (Neem oil) has been reported [13] to have several health and medicinal effects on humans. Detailed accounts on the exact effect it poses have sprung arguments in the research community. While some claim they are negative, other dogma see it from the beneficial point. This study was engineered to find the exact effect that Neem seed oil will pose to male reproductive gamete; specifically the semen quality and the possible contribution of lipid peroxidation. This study has shown that neem seed oil caused reduction of semen quality (motility, morphology and count). This is in line with the findings earlier reported by Sinha, *et al.* [14], Kastura and Ahmad [15], Sharma and Sairam [16], and Bardhan, *et al.* [17].

Results show that with increase in the quantity of neem seed oil, semen quality (motility) was negatively affected with increase in oxidative stress (tables 1.1 and 1.2) above. The study also found, that semen quality, oxidative stress on it, and incubation periods were directly proportionate to one another. (fig 1.2 and 1.3) above. Upon statistical analysis, Malonaldehyde (MDA) concentration in samples with increased quantity of neem seed oil was significantly high at $p<0.05$ (table 1.1). All these were in comparisons with the control group. Also, Malonaldehyde concentrations in samples with same quantity of neem seed oil that was incubated at 37º C for 10, 15, 20 minutes was significantly high; with $p < 0.05$ compared with control group (fig 1.3).

The reason for the above could have been that, Neem seed oil has possibly caused an oxidative stress on sperm cells, resulting in exudation of cellular contents and possibly death (Apoptosis), hence a reduction in sperm quality.

5. Conclusion

Within the ambient of vulnerability to human, logical and experimental errors, The study demonstrated the spermicidal effect of need seed extract through lipid peroxidation of cell membrane of sperm cells.

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