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Effect of Supplementation with Moringa Oleifera on Antioxidant and Oxidative Stress Biomarkers of Infertile Women: A Pilot Open-Label Case-Control Randomized Clinical Study

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Abstract

Background: Excessive generation of free radicals has been reported to be associated with infertility. Clinical studies investigating the effect of supplementation with *moringa oleifera* on antioxidant and oxidative stress markers of infertile women are depleted. This study aims to investigate the effects of supplementation with *Moringa Oleifera* on the antioxidant and oxidative stress markers of infertile women.

Methodology: This study was an open-label case-control randomized clinical study. A total of 100 women, aged 35-50 years, attending Island Maternity Hospital, Lagos, and diagnosed with infertility were recruited and screened. Pre-enrollment fertility screening for LH, FSH, Prolactin, and E2 was measured using the ELISA technique. Of these numbers, 40 had elevated reproductive hormones and were excluded. Sixty (60) of these infertile women with normal hormone levels were randomized into two groups - cases and controls, each comprising 30 subjects respectively. Thirty (30) fertile women were recruited for comparison of the baselines of biomarkers between fertile and infertile women. A five-milliliter (5ml) blood sample was collected and used to assess the baseline parameters. The case group was supplemented with 2g of Moringa capsules daily for four weeks. The control group received no supplementation. After four weeks post-supplementation, Plasma levels of Malondialdehyde (MDA), Total Plasma Peroxide (TPP), Lipid hydroperoxide (LPO), Total Antioxidant Capacity (TAC), Glutathione reductase (GR), and Glutathione (GSH) were reassessed using standard methods. The Oxidative Stress Index (OSI) was calculated using an approved conventional method.

Results: The mean levels of markers of oxidative stress (MDA, TPP, LPO, and OSI) were higher in infertile women than in the fertile group. However, only TPP and OSI were significantly higher ($p \leq 0.05$). Levels of MDA, TPP, LPO, and OSI were lower in the supplemented group than in those unsupplemented ($P>0.05$). The levels of antioxidant biomarkers (TAC, GR, and GSH) were slightly higher in the infertile women supplemented with Moringa Oleifera than in the unsupplemented group ($P>0.05$).

Conclusion: This study revealed that an increase in oxidative stress biomarkers is associated with female infertility. Supplementation with *moringer oleifera* in infertile women could help reduce the effects of OS and may likely improve pregnancy outcomes.

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Introduction

The World Health Organization defines infertility as the inability of a couple to achieve pregnancy after one year of uninterrupted sexual intercourse (Chowdhury *et al., 2017*). Estimates from 1997 suggested that worldwide, about five percent (5%) of all heterosexual couples had unresolved infertility (Himmel *et al.,* 1997). Male infertility constitutes about 20% - 30% of infertility cases, while 20% - 35% are due to female infertility and 25% - 40% are due to combined problems in both sexes (Chowdhury *et al., 2017).*

There are basically two types of infertility: primary and secondary. While primary infertility refers to a condition in which couples have been unable to achieve conception in spite of several sexual intercourse episodes, secondary infertility refers to a condition where couples have been able to achieve conception at least once, but find it difficult to achieve another in spite of several trials.

Several factors have been identified as causes of infertility in both sexes. These include abnormal anatomical structures in reproductive organs, hormonal disorders, and genetic factors (Mauricio and Ludovico, 2013; Sang *et al*., 2023). Other causes which have been implicated are infections, Cushing's syndrome, hyperprolactinaemia, thyroid disorders, polycystic ovary syndrome (PCOS), and primary ovarian insufficiency (POI) (Michael *et al*., 2021, Sang *et al*., 2023). Furthermore, ovulatory problems which generally manifest with sparse or absent menstrual periods have been implicated as one of the major causes of infertility among women (Chowdhury *et al., 2017).*

Metabolic processes in humans result in the generation of reactive oxygen species (ROS). The antioxidant system of the body provides protection against the damaging effects of reactive oxygen species (ROS). The imbalance between ROS production and antioxidant defense leads to oxidative stress (OS). The harmful effect of ROS is neutralized by a broad class of protective agents termed antioxidants, which prevent oxidative damage by reacting with free radicals before any other molecule can become a target. Antioxidants are probably now regarded as superheroes for maintaining health. The

enzymatic antioxidants such as Superoxide Dismutase (SOD), Catalase (CAT), Glutathione Peroxidases (GPx), and non-enzymatic antioxidants like Vitamin C, Vitamin E, Ceruloplasmin, albumin, and reduced glutathione (GSH) play important roles in the protection of cells against free radical damage (Halliwel, 2001; Chowdhury *et al., 2017;* Tarique Hussain *et al.*, 2021)).

Excessive generation of ROS, higher than the supply of antioxidants to the body system, results in oxidative stress (OS). It has been reported that OS dysregulates the balance between reactive oxygen species (ROS) and the antioxidant system in the body (Tarique Hussain *et al.*, 2021).

Evidence abounds on the role of ROS in female reproduction. Studies on human and animal models have shown that ROS influences oocyte development, maturation, follicular atresia, corpus luteum function, and luteolysis (Agarwal et al., 2005). The ROS affects follicular function, development of oocytes, endometrium, and the normal reproductive environment needed to achieve effective function (Agarwal and Gupta, 2005; Manika *et al.*, 2017). The luteinizing hormone (LH) surge precipitates the upregulation of ROS that affects all other major reproductive functions, including oocyte maturation, ovarian steroidogenesis, corpus luteal function, and luteolysis (Manika *et al.,* 2017). The availability of an adequate anti-oxidant system balances ROS generation and maintains the cellular functions, permitting a normal reproductive environment. Both enzymatic and non-enzymatic antioxidants, namely, vitamins and minerals, are present in the follicles, and these protect the oocytes from ROS damage. The overproduction of ROS results in oxidative stress, and this has an effect on the quality of oocytes which might result in anovulation (Manika *et al.*, 2017).

Excess ROS in the follicle may overwhelm the follicular fluid antioxidant defense mechanism, thereby damaging the oocytes. Furthermore, severe OS could precipitate the damage of the DNA of oocytes and spermatozoa, thereby leading to defective fertilization; and when fertilization is achieved, OS-induced apoptosis, embryo fragmentation, implantation failure, abortion, impaired placentation, and congenital abnormalities could occur (Manika *et al.*, 2017; Tarique Hussain *et al.*, 2021).

The use of plant-based products for food and medicinal purposes has been a regular practice in Nigeria and Africa for many centuries and has become a key component of the healthcare delivery system. Studies have shown that over 80% of populations in developing countries have used herbal supplements as a primary source of healthcare; and in Nigeria, over 90% of the rural population and over 40% of the urban settlers depend on herbal-based products to meet their healthcare needs (WHO, 2013; WHO, 2022; Onyeaghala *et al.*, 2023).

Moringa oleifera (M. oleifera) is a tree that grows on most continents, including Africa, Asia, America, and many other parts of the world. It is called by different names, including: drumstick, which is due to the appearance of its immature pods; the horseradish tree and the ben oil tree; the 'tree of life' or the 'Miracle Tree' due to its economical importance and versatility [Sidney et al., 2015]. Its leaves, roots, stems, and flowers have been widely used in traditional medicine and have been reported to possess diverse medicinal properties [Razis *et al.*, 2014]. Moringa oleifera has been reported to possess anti-inflammatory, antihypertensive, diuretic, antimicrobial, antioxidant, anti-diabetic, anti-hyperlipidemic, antineoplastic, antipyretic, antiulcer, cardioprotective, and hepatoprotective properties [Razis *et al.*, 2014; Sidney *et al.*,

2015; Plott, 2017]. The numerous health benefits derivable from moringa oleifera have been attributed to the diverse chemicals that are present in the medicinal plant. The leaf of moringa oleifera has been reported to contain 17 times more calcium than that of milk and 10 times more Vitamin A than that of carrots [Plott, 2017].

Mammalian reproductive physiology is regulated by the gonadotropins - luteinizing hormone (LH) and follicle-stimulating hormone (FSH) - secreted from the anterior pituitary, which act on the gonads to produce sex steroids. The gonadotropins are controlled by gonadotropin-releasing hormone (GnRH), secreted in a pulsatile manner from the hypothalamus. This reproduction is highly controlled by the hypothalamic-pituitary axis. Interruption of these processes at any functional event in either sex leads to fertility impairment (Tsutsumi *et al*., 2009). Herbal therapy that has actions on the hypothalamus-pituitary-gonadal axis may influence reproductive physiology and ameliorate some infertility problems (Wang et al., 2017; Sulagna and Pallav 2018). Thus, the identification of efficacious herbal plants that influence the hypothalamic pituitary-gonadal axis may provide alternative treatments for infertility and ultimately lead to more effective medicine. A number of recent studies have identified plants that have fertility enhancement (and *Moringa oleifera* may be one of such plants, given its capacity in in-vivo studies in laboratory animals to inhibit inflammation and oxidative stress (Lans *et al.,* 2018; Ben *et al.,* 2023).

There is a dearth of clinical studies and information about this plant on infertility. Premised on this, there is a need to properly evaluate and document its clinical usefulness among women diagnosed with primary infertility. This study is therefore aimed to study the effect of supplementation of *Moringa oleifera* on the antioxidant and oxidative stress markers of infertile women.

Methodology

Study Design: This study was an open-label case-control randomized clinical study.

Ethical Issues: Ethical approval was obtained from the Lagos State Ministry of Health, Lagos State Ikeja. Informed consent was obtained from all participants. The study followed the principles declared in the Helsinki Declaration and Good Clinical Practice (GCP) regulations.

Study Population: A total of one hundred (100) women, attending Island Maternity Hospital, Lagos Island, diagnosed with infertility, were recruited and screened for inclusion in the study. The fertility hormonal profile – LH, FSH, Prolactin, and E2 - was measured. Of these numbers, 40 had an elevated reproductive hormonal profile and were excluded. Sixty (60) of these infertile women had a normal hormonal profile. These women were screened and randomized into two study groups (Figure 1), and randomization was performed by the tossing of a coin.

Group 1. They comprised 30 women diagnosed with primary infertility who met all the required inclusion criteria. The women had no evidence of hormonal abnormalities, and there was no known cause of infertility evidenced by available medical records, clinical history, and laboratory findings. A blood sample was collected, and the baseline levels of oxidative stress and antioxidant biomarkers were determined. Thereafter, the subjects were supplemented with 2g of

moringa capsules daily for four weeks.

Group 2: This was the control group. They comprised 30 women who were diagnosed with infertility. The women had no evidence of hormonal abnormalities, and there was no known cause of infertility evidenced by available medical records, clinical history, and laboratory data. They received no supplementation.

Thirty (30) women with established fertility were recruited to establish the fundamental relationship between the variables and fertile and infertile women within this environment. This group served as the positive control group. They comprised 30 fertile women who also attended the maternity hospital. They were included in the study in order to establish a baseline relationship between markers of oxidative stress and antioxidants between infertile and fertile women. They received no supplementation.

Inclusion Criteria

Subjects were recruited into the study if they fulfilled the following conditions:

- Age 35-50 years
- No elevated levels of the reproductive hormone profile
- No history of chronic myocardial infarction, liver disease, or diabetes
- Not on any antioxidant medication or supplement in the last six months

Exclusion Criteria

- Women who had undergone hysterectomy
- Women on hormonal replacement therapy
- History and evidence of chronic disease
- Abnormal hormonal profile

Sample Size Determination

$N= 2SD^2 [Z+P]^2/d^2$ (Jaykaran and Biswas,2013)

N= number of samples required per group, SD = Standard deviation of the end point

Z = Confidence interval at 95%, which is 1.96

P= Statistical power at 80%, which is 0.84

d = difference between the mean measurements.

For this study:

SD= 15 (assumed and acceptable SD for most laboratory measurements)

Z = 1.96 (statistical value at a 5% error margin)

P= 0.84 (statistical table at 80% power)

d =15% (assume that a 15% reduction or increase in measurable analytes is considered clinically significant)

Applying the formula: $N= 2SD^2 [Z+P]^2/d^2$

$N= 2[15]^2[1.96+0.84]^2/ 15^2$

N= 450 [7.84]/225

N= 15.68= 16

Considering 20% attrition (3.2), the minimum number of patients required for the study per group is (16+ 3.2= app 20). 30 subjects per group

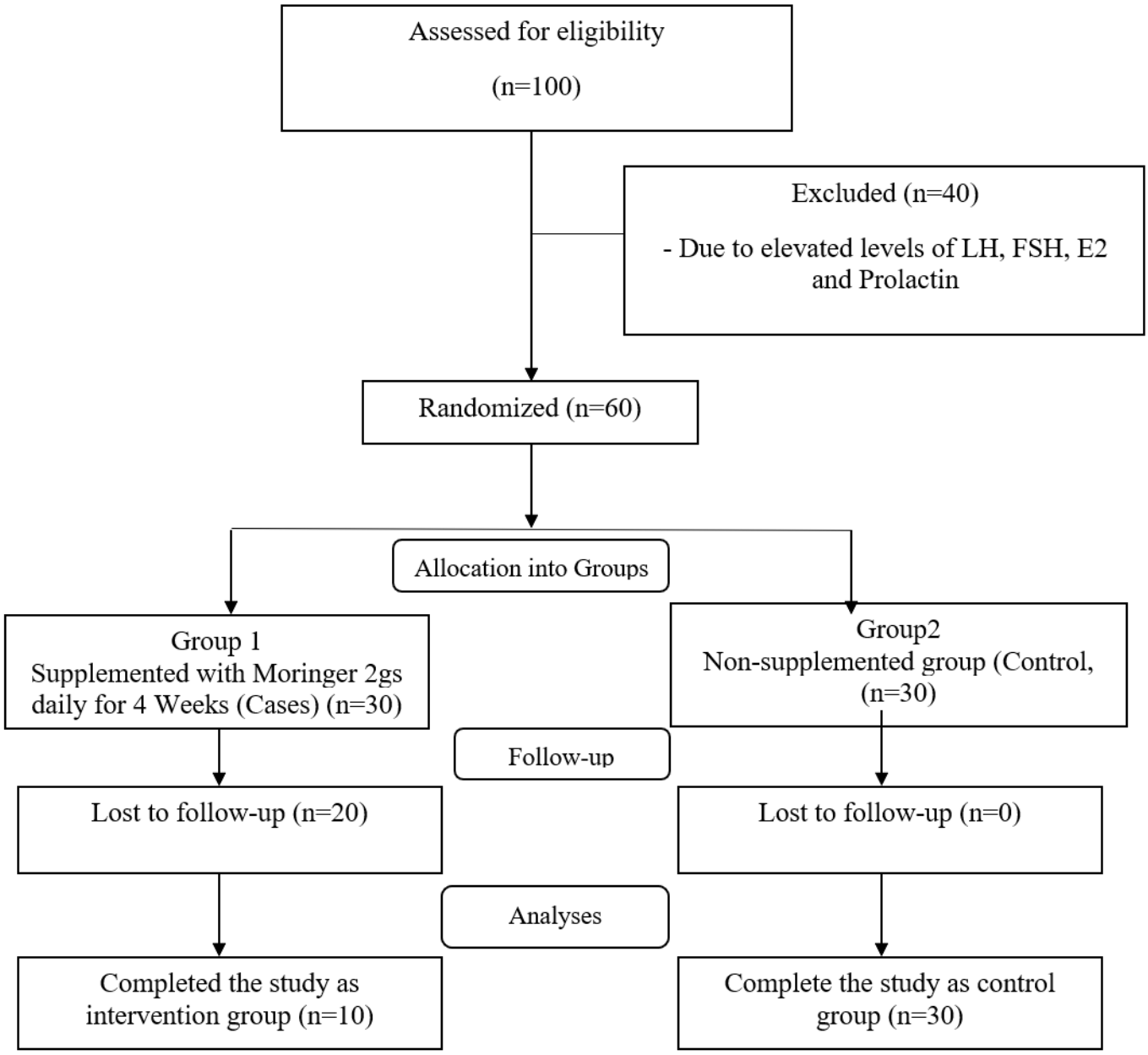

Figure 1. Study Flow Chart (CONSORT)

Collection of Blood Samples:

Five milliliters (5ml) of venous blood was collected into a lithium heparin bottle. This was spun, and the plasma was stored at -20^{o}C until ready for use.

Purchase of Supplement: *Moringa Oleifera* (MO) supplement was purchased from Afe Babalola University. The University engages in the massive production of *Moringa Oleifera* capsules for commercial purposes. The production of MO is under well-regulated Good Manufacturing Practice (GMP) and quality assurance. The company has Good Manufacturing Practice (GMP) certification with NAFDAC. The supplement produced at the company is registered with NAFDAC. The supplement was stored under the recommended temperature throughout the duration of the study, and the storage temperature was strictly monitored.

Supplement Dispensing and Accountability. Subjects were given packs of the supplement which would last them for four weeks as detailed in the protocol. They were expected to take 2g of the supplement daily in a divided dose of 1g in the morning and 1g in the evening after meals, respectively. They were advised to continue with their normal lifestyle and should not take any other supplement other than the *Moringa Oleifera*. The study physician followed up with the patients on the phone once every week to inquire about their compliance with the intake of the supplement and other health conditions. Compliance was ascertained by requesting the subjects to count the number of supplements left in the bottle dispensed to them during the follow-up visit.

Laboratory Methods

Measurement of Malondialdehyde (MDA) was based on the method of Adam-Vizi and Seregi (1982). The principle of the test is based on the reaction between 2-thiobarbituric (TBA) and MDA. On heating in an acidic medium, a pink complex is formed which absorbs maximally at 532nm, the absorbance being directly proportional to the concentration of MDA present in the sample.

Determination of Total Plasma Peroxide (TPP) was performed using the Fox-2 reagent as described by Miyazawa, 1989, with minor modifications by Harma et al. (2003). The principle of the test is based on the oxidation of ferrous ion (Fe2+) to ferric ion (Fe3+) by various types of peroxides contained within the plasma samples to produce a colored ferric–xylenol orange complex whose absorbance was measured at 560nm. The total plasma peroxide content of the sample was determined as a function of the difference in absorbance between the test tube and the blank tube using a solution of H2O2 as the standard (100mM-H2O2).

Lipid Hydroperoxide (LPO) was measured based on the method described by Nourooz-Zadeh et al. (1995). The principle is based on the rapid peroxide-mediated oxidation of Fe^{2+} to Fe^{3+} under acidic conditions. The latter, in the presence of Xylenol Orange, forms a Fe-Xylenol Orange complex which was measured spectrophotometrically at 560 nm.

Determination of Total Antioxidant Capacity (TAC) was carried out using the Ferric Reducing Antioxidant Powder (FRAP) reagent as described by Koracevic et al., (2001).

At low pH, the 2,4,6-tripyridyl-s-triazine (TPTZ) ferric complex is reduced to the ferrous form (which has an intense-blue color). The color change can be monitored by measuring the change in absorbance at 593nm. Any half reaction that has a lower redox potential under the reaction conditions than that of the ferric–ferrous half reaction will drive the ferrous formation. The change in absorbance is therefore directly related to the combined or total reducing power of the electron-donating antioxidants present in the reaction mixture.

Total Glutathione was measured using the method described by (Anderson, 1985). The method is based on the reaction of GSH with the thiol reagent DTNB (5,5'-dithiobis (2-nitrobenzoic acid)) to form GSSG and TNB(5-thionitrobenzoic acid), which is then detected spectrophotometrically at 412nm. Glutathione disulfide (GSSG) is the oxidized form of glutathione. It is reduced to GSH in the presence of NADPH by the glutathione reductase (GR). The glutathione peroxidase (GP)

converts hydrogen peroxide to water.

The oxidative stress index (OSI), an indicator of the degree of oxidative stress, was calculated using the method described by Harma et al. (2003). It was calculated from the values of TPP and TAC using the formula (TPP/TAC x 100).

The estimation of the Glutathione Reductase Assay was as described by Teitze (1969). The assay is based on the spectrophotometric determination of glutathione reductase activity by the increase in absorption caused by the reduction of dithiobis (2-nitrobenzoic acid) (DTNB) at 412 nm.

Data Analysis

Statistical Package for the Social Sciences (SPSS) software version 21.0-compatible software was used for the analysis of data. A Student t-test was used for comparison of variables. The significance level for variables was set at $P<0.05$. The data was expressed as the mean ± standard deviation of the mean.

Results

The baseline data of markers of oxidative stress and antioxidants of both fertile and infertile women are shown in Table 1. The mean levels of Total Plasma Peroxidase (TPP) and the Oxidative Stress Index (OSI) were significantly lower in fertile women than in the infertile group ($P< 0.05$).; and the mean level of Glutathione Reductase (GR) was also significantly lower in fertile women than in the infertile group ($P< 0.05$).

Table 2 shows the data of markers of oxidative stress and antioxidants for both infertile women and infertile women post supplementation with *Moringa Oleifera*. It was evident that the mean levels of markers of oxidative stress were lower in infertile women who were supplemented with *Moringa Oleifera* than those unsupplemented. However, the difference in the mean was not significant ($P>0.05$). The mean level of all antioxidant biomarkers was higher in the infertile women supplemented with Moringa Oleifera than in the unsupplemented group, but the difference in the mean was also not significant ($P>0.05$).

Table 1. Comparison of baseline data between infertile and fertile women (n=60)

Parameters	Infertile Women (n=30)	Fertile Women (n=30)	t-test	p-value
MDA (uM/cm)	2.49 ± 1.45	2.08 ± 0.71	1.421	0.161
TPP (um/L)	69.14 ± 31.42	41.13 ± 8.73	4.848	0.000*
LPO (um/L)	17.88 ± 9.28	17.15 ± 3.97	0.409	0.684
OSI	11.16 ± 8.93	6.07 ± 2.31	3.112	0.003*
TAC (um/L)	724.02 ± 217.09	731.68 ± 187.79	0.240	0.811
GR (um/L)	168.97 ± 70.36	290.78 ± 60.53	-7.322	0.000*
GSH (uM/L)	315.40 ± 67.74	316.22 ± 90.42	-0.040	0.968

*N = 60 *p<0.05 (Significant).*

Table 2. Comparison of data between group 1 (infertile women) and Group 2 (infertile women post supplementation, N=40

Parameters	Infertile women (n=30)	Infertile women post supplementation) (n=10)	t-test	p-value
MDA (uM/cm)	2.49 ± 1.45	2.30 ± 1.00	-0.330	0.743
TPP (um/L)	69.14 ± 31.42	58.57 ± 34.31	-1.656	0.106
LPO (um/L)	17.88 ± 9.28	15.71 ± 10.91	-0.234	0.816
OSI	11.16 ± 8.93	9.12 ± 8.62	-0.915	0.366
TAC (um/L)	724.02 ± 217.09	736.13 ± 253.02	0.096	0.924
GR (um/L)	168.97 ± 70.36	196.90 ± 54.84	-1.142	0.261
GSH (uM/L)	315.40 ± 67.74	320.50 ± 94.64	-0.186	0.853

*N = 40, *p<0.05 (i.e. Significant).*

Discussion

Infertility, defined as the inability of couples to achieve reproduction after one year of uninterrupted sexual intercourse, has become a global health issue. Infertility is a complex problem, and various factors have been identified to be responsible. The role of oxidative stress in the pathogenesis of various health disorders, including infertility, has been reported, and the neutralization of oxidative stress might help to improve pregnancy outcomes.

The findings from this study showed that the mean levels of markers of oxidative stress (MDA, TPP, LPO, and OSI) were all higher in the infertile group than in the fertile group. However, only the mean levels of TPP and OSI were statistically significant when compared with those of fertile women. On the other hand, antioxidant biomarkers (TAC, GR, and GSH) were all higher in the fertile women than in the infertile group. This observation adds credence to the idea that OS plays a significant role in female infertility.

The findings from this study are consistent with the study of Sara*et al.,* (2015), who reported reduced and higher levels of MDA and TAC in infertile and fertile women, respectively. However, the study of Sara *et al*., (2015), reported a non-

significant level in MDA among infertile women, but a significant level in TAC among the fertile group. The non-statistically significant levels reported in the plasma levels of MDA and TAC in the study group could be due to the sample size used for the study.

Reactive oxygen species (ROS) impact female reproductive function, and antioxidants serve as protective systems to the effects of oxidative stress. The role of plasma concentrations of ROS in folliculogenesis, maturation of oocytes, and normal uterine function has been established by a previous study (Agarwal et al., 2008). Excessive production of free radical biomarkers has been reported to affect the normal reproductive process (Agarwal et al., 2005). The increased level of the biomarkers of oxidative stress reported in the infertile women in this study confirms that oxidative stress plays a role in the normal reproductive process. Available evidence has confirmed the important interplay between ROS and the antioxidant system in ovulation, fertilization, steroidogenesis, and endometrial receptivity (Brzezinski, 1987; Ben et al., 2023)

In this study, after supplementing infertile women with MO, antioxidant biomarkers were higher in the infertile women than in the control group, but the difference observed was not significant ($P > 0.05$). The lack of significance in the levels observed might be attributed to the small sample size used for the analysis. Due to economic challenges precipitated by the COVID-19 pandemic, many patients who were supplemented with MO were not able to return for post-supplementation sample collection. This could account for the high loss to follow-up observed in this study.

Documented clinical data on the use of MO in infertile women is depleted; however, there is evidence suggesting the use of natural products as possible alternatives to conventional drugs in the management of infertility (Seungjin *et al.*, 2020). Natural products are constantly being investigated to unravel their health benefits, including their role in limiting oxidative stress and enhancing the body's antioxidant defense system. *Moringa Oleifera*, a well-consumed herbal supplement, is rich in bioactive compounds, micronutrients, macronutrients, carbohydrates, proteins, functional peptides, vitamins, minerals, and essential amino acids, as well as a number of glycosides. The increase in the level of antioxidants observed in the infertile women supplemented with MO could be attributed to the bioactive contents of MO. Furthermore, the flavonoids from the *Moringa Oleifera* leaf have been documented to increase the activities of superoxide dismutase, catalase, and glutathione peroxidase and decreased the Malondialdehyde activity and intracellular accumulation of reactive oxygen species (ROS) (Ji *et al.*, 2020). All these could result in a reduced generation of free radicals with an increase in antioxidant biomarkers.

Conclusion

This study has shown that infertile women, when supplemented with*Moringa oleifera*, have varying levels of oxidative stress and antioxidant biomarkers. Supplementation with *Moringa oleifera* in infertile women could help in reducing the effects of OS, thereby improving pregnancy outcomes.

Recommendations and further study

Considering the mental and social consequences of infertility, integrating a non-invasive and inexpensive method in the management of infertility with a view to achieving pregnancy among infertile women could be very helpful. Premised on this, we recommend large population-based clinical trials to generate more data.

Limitations of the study

One of the major limitations of this study is the high loss to follow-up reported. Future studies should be designed with a view to using statistical models to manage excessive loss to follow-up.

References

- Adam Vizi, V. and Seregi, M. (1982) Receptor Dependent Stimulatory Effect of Noradrenaline on Na+/K+ ATPase in Rat Brain Homogenate. Role of Lipid Peroxidation. Biochemical Pharmacology, 31, 2231-2236. http://dx.doi.org/10.1016/0006-2952(82)90106-X
- Agarwal A., Gupta S. and Sharma R. (2005). Oxidative stress and its implications in female infertility – a clinician's perspective. *Reproductive Biomedicine Online,* 11: 641-650.
- Agarwal A., Gupta S. and Sharma R. (2005). Role of oxidative stress in female reproduction.*Reproductive Biomedicine online*, 3(28):1477-7827.
- Agarwal A., Makker K. and Sharma R. (2008). Clinical relevance of oxidative stress in male factor infertility: an update. *American Journal of Reproductive Immunology,* 59: 2-11.
- Anderson, Mary E (1985). [70] Determination of glutathione and glutathione disulfide in biological samples, Methods in Enzymology, Academic Press, Volume 113: 548-555, https://doi.org/10.1016/S0076-6879(85)13073-9.
- Ben Enoluomen Ehigiator, Uchechukwu Loveth Iyanyi , Kelechi Samuel Mobisson , Onuchkwu Imelda Ukata (2023). In vivo and in silico investigation of effects of ethanol extract of Moringa oleifera leaves on female fertility, using fruit flies and molecular docking. Journal of Clinical and Basic Research, 2023, Volume 7, Number 3 pp 1-6
- Brzezinski A., Seibel M.M., Lynch H.J., Deng M.H. and Wurtman R.J. (1987). Melatonin in human preovulatory follicular fluid. *Journal of Clinical Endocrinology and Metabolism*, 64: 865-867.
- Chowdhury S.H., Cozoma A.I., Chowdhury J.H (2017). Infertility essentials for the Canadian medical licensing exam. Review and prep for MCCQE part 1,2nd edition:wolters Kluwer Hong kong
- Halliwell B., (2001). Role of free radicals in neurodegenerative diseases: therapeutic implications for antioxidant treatment. *Drugs & Aging*, 18:685–716.
- Harma M, Harma M, Erel O (2003) Increased oxidative stress in patients with hydatidiform mole. Swiss Med Wkly. 133:563–566.
- Himmel W, Ittner E, Kochen MM, Michelmann HW, Hinney B, Reuter M, Kallerhoff M, Ringert RH (1997). "Voluntary Childlessness and being Childfree". *British Journal of General Practice,* 47 (415): 111–8.
- Ji Liu, Guangming Ma, Yan Wang, and Yonggen Zhang (2020). Moringa Oleifera leaf flavonoids protect bovine

mammary epithelial cells from hydrogen peroxide-induced oxidative stress in vitro. Reproduction in Domestic Animals. 55(6):711-719.

- Koracevic D, Koracevic G, Djordjevic V, et al (2001). Method for the measurement of antioxidant activity in human fluids. J Clin Pathol. 54:356–361.
- Lans Cheryl, Lisa Taylor-Swanson, Rachel Westfall (2018) Herbal fertility treatments used in North America from colonial times to 1900, and their potential for improving the success rate of assisted reproductive technology. Reproductive Biomedicine & Society Online. Volume 5,2018, Pages 60-81. https://doi.org/10.1016/j.rbms.2018.03.001
- Manika Kala, Muhammad Vaseem Shaikh, Manish Nivsarkar (2017) . Equilibrium between anti-oxidants and reactive oxygen species: a requisite for oocyte development and maturation. Reprod Med Biol. 16(1): 28–35. doi: 10.1002/rmb2.12013
- Mauricio S. Abrao and Ludovico Muzzi (2013). Anatomical causes of female infertility and their management. *International Journal of Gynaecology and Obstetrics,*123: S2
- Michał Ciebiera, Sahar Esfandyari, Hiba Siblini, Lillian Prince, Hoda Elkafas, Cezary Wojtyła , Ayman Al-Hendy , Mohamed Ali (2021). Nutrition in Gynecological Diseases: Current Perspectives. Nutrients, 13(4):1178. doi: 10.3390/nu13041178
- Miyazawa T. (1989) Determination of phospholipid hydroperoxides in human blood plasma by a chemiluminescence-HPLC assay. Free Radic Biol Med. 7:209–217.
- Nourooz-Zadeh Jaffar, Javad Tajaddini-Sarmadi and Simon P. Wolff (1995). Measurement of Hydroperoxides in Edible Oils Using the Ferrous Oxidation in Xylenol Orange Assay.J. Agric. Food Chem. 43(1): 17–21
- Onyeaghala Augustine Anayochukwu, Arinze Favour Anyiam, Danladi Chiroma Husaini, Emmanuella Ogechi Onyeaghala, Ejeatuluchukwu Obi. (2023). Herbal supplements as treatment options for COVID-19: A call for clinical development of herbal supplements for emerging and re-emerging viral threats in Sub-Saharan Africa. Scientific African, Volume 20,2023, e01627, https://doi.org/10.1016/j.sciaf.2023.e01627
- Plott, E.G (2017). Moringa Helps My Energy While I Go Through Menopause. Available at: http://plottpalmtree.miiduu.com/menopause accessed on 8th August, 2023
- Razis AFA, Ibrahim MD, Kntayya SB (2014). Health benefits of Moringa oleifera. Asian Pac J Cancer Prev:. DOI:10.7314/ APJCP 20: 8571
- Sang Q, Ray PF, Wang L. (2023). Understanding the genetics of human infertility
- Sara Soleimani Rad, Shamsi Abbasalizadeh., Amir Ghorbani Haghjo., Mehzad Sadagheyani1 M.D., Azadeh Montaseri and Jafar Soleimani Rad (2015). Evaluation of the melatonin and oxidative stress markers level in serum of fertile and infertile women. *Iranian Journal of Reproductive Medicine*, 13(7):439-444.
- Science. 2023 380(6641):158-163. doi: 10.1126/science.adf7760.
- Seungjin Noh, Ara Go, Da Bin Kim, Minjeong Park, Hee Won Jeon and Bonglee Kim (2020). Role of Antioxidant Natural Products in Management of Infertility: A Review of Their Medicinal Potential. *Antioxidants* 9:957.
- Sidney J. Stohs and Michael J. Hartman. Review of the Safety and Efficacy of Moringa oleifera. Phytother. Res. 2015; 29: 796–804
- Sulagna Dutta and Pallav Sengupta (2018). Medicinal herbs in the management of male infertility. J Pregnancy

Reprod, 2018 doi: 10.15761/JPR.1000128Volume 2(1):1-6

- Tarique Hussain, Ghulam Murtaza Elsayed Metwally Dildar Hussain Kalhoro , Muhammad Saleem Kalhoro , Baban Ali Rahu, Raja Ghazanfar Ali Sahito, Yulong Yin , Huansheng Yang, Muhammad Ismail Chughtai , Bie Tan (2021) The Role of Oxidative Stress and Antioxidant Balance in Pregnancy. Mediators. Inflamm doi: 10.1155/2021/9962860. eCollection 2021
- Tietze Frank (1969). Enzymic method for quantitative determination of nanogram amounts of total and oxidized glutathione: Applications to mammalian blood and other tissues. Analytical Biochemistry, Volume 27, Issue 3, 1969, https://doi.org/10.1016/0003-2697(69)90064-5.
- TSUTSUMi Rie and NicholaS J.G. WeBSTeR. (2009). GnRH Pulsatility, the Pituitary Response and Reproductive Dysfunction. Endocrine Journal. 56 (6): 729-737
- Wang S., He G., Chem M., Zuo T., Xu W., Liu X. (2017). The role of antioxidant enzymes in the ovaries*Oxidative Medicine and cellular longevity*, Vol 2017, Article ID 4371714, 14 pages.
- Word Health Organization, (2013). WHO traditional medicine strategy: 2014-2023. https://www.who.int/publications/i/item/9789241506096 (accessed August, 24, 2023)
- World Health Organization. Coronavirus (COVID-19) Dashboard. (2022). Available athttps://covid19.who.int/. Accessed 23August, 2023.