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Azadirachta indica A. juss: Safety and Efficacy during Pregnancy and Lactation

Nkechi Enwerem[1*] and Samson Amos[2]

[1]*College of Nursing and Allied Health, Division of Nursing, Howard University, Washington DC, USA.*

[2]*Department of Pharmaceutical Sciences, Cedarville University, USA.*

***Correspondance:**
Nkechi Enwerem, College of Nursing and Allied Health, Division of Nursing, Howard University, Washington DC, USA, E-mail: nkechi.enwerem@howard.edu.

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ABSTRACT

Background: *In Nigeria decoctions and aqueous extract of Azadirachta indica A. juss (AI), are commonly used in the treatment of malaria. Some women have been observed consuming aqueous extract of AI during pregnancy and lactation because of the folkloric belief that it is potentially harmless.*

Objectives: *There is a paucity of data on the effects of consumption of AI during pregnancy and lactation. Its use by women during lactation and pregnancy, calls for an in-depth understanding of its efficacy and potential for harm during pregnancy and lactation.*

Methodology: *AHMED, CINAHL, Cochrane CENTRAL, Cochrane Library, Medline, Internet journals, Natural Medicines comprehensive database, and Natural standard were reviewed from inception to 2017 for information on Azadirachta indica A. juss , as it relates to its use on "pregnancy", "lactation", and "breastfeeding". The Latin and common name of Azadirachta indica A. juss (AI), were also used as keywords in the search. Data were compiled based on the grade and evidence found.*

Results: *There were no evidence based scientific data to support the use of Azadirachta indica A. juss during pregnancy and lactation. However, there is invitro evidence that pregnancy termination was observed in rodents and primates given neem extract at early post-implantation stage. No residual permanent effect and fertility was regained following treatment in subsequent cycles. In lactating rats AI at concentrations of 400 mg/kg and 600 mg/kg significantly increased in a dose dependent manner the packed cell volume (PCV), red blood cell (RBC), and a decrease in blood glucose level. AI has the potential to interact with some prescription medications such as antidiabetic drugs.*

Conclusions: *A more rigorous and well controlled- clinical research is needed before this plant can be used during pregnancy and lactation. It is important for consumers and clinicians to know that AI can lower blood sugar level. This is particularly important in the developing tropical countries where AI is used for the management of malaria. It is equally important for male and female in their reproductive stage to know that AI has some effect on fertility.*

Introduction

In Africa, about 80 % of its population use herbal medicine for management of different ailments [1]. Medicinal plants play a key role in traditional health care system of developing countries such as Nigeria. With this enormous use, most of these plants lack information on standardization, active chemical constituent, quality, clinical studies, safety and efficacy.

Azadirachta indica A. juss (AI) is a large evergreen tree belonging to the genus, Azadirachta and a member of the mahogany family

Meliaceae. It is commonly known as neem, nimtree, Indian lilac, and in Nigeria, it is popularly called "Dogonyaro" [1]. It is originally grown in India and its subcontinent where it is known as the "Village Pharmacy" because of its healing usefulness. It has been used in Ayurvedic medicine for more than 4000 years [2]. The latinized name of Neem – *Azadirachta indica* – is derived from the Persian: Azad = Free, dirakht = Tree, AI is cultivated in tropical and semi-tropical regions including Nigeria [3].

Phytochemical analysis of neem reveals the presence of alkaloids, limonoids (a terpenoid), quercetin (a flavonoid) and nimbosterol (β-sitosterol), triterpenes [4,5]. Other constituent of neem leaves include protein, carbohydrates, minerals, calcium, phosphorus, vitamin C, carotene, glutamic acid, amino acids and several fatty acids [6,7].

In traditional medicine a decoction made from the bark, leaf, root, fruits and flowers is used in the treatment of a variety of ailments including blood morbidity, biliary afflictions, and itching, skin [1]. Apart from their traditional uses, there are several reports on the biological activities and pharmacological actions of AI based on modern scientific investigations (Table 3). In rats, different parts of *Azadirachta indica* have been shown to produce significant antioxidant, cancer chemoprotective and hepatoprotective, effects [8-10]. Gedunin a compound isolated from AI, has antimalarial activity [11]. Seeds and leaves of *Azadirachta indica*, are effective against AI resistance and sensitive strains of malaria [12].

In Nigeria decoctions and aqueous extract of AI are commonly used in the treatment of malaria. Some women have been observed consuming aqueous extract of *Azadirachta indica* during pregnancy and lactation because of the folkloric belief that it is potentially harmless [1].

There is however, paucity of data on the effects of consumption of AI during pregnancy and lactation. Its use by women during lactation and pregnancy, calls for an in-depth understanding of its efficacy and potential for harm during pregnancy and lactation.

One of the ways to address these issues is to do a systemic review of the literature. In this article, we will review the effectiveness of *Azadiracta indica* with emphasis on issues related to pregnancy, lactation and breast feeding. This paper is the first on the evaluation of the efficacy and safety of AI during pregnancy and lactation.

Methods

AHMED, CINAHL, Cochrane CENTRAL, Cochrane Library, Medline, Internet journals, Natural Medicines comprehensive database, and Natural standard were reviewed from inception to 2017 for information on *Azadirachta indica* A. juss , as it relates to its use on “pregnancy”, “lactation”, and “breastfeeding”. The Latin and common name of *Azadirachta indica* A. juss (AI), were also used as keywords in the search.

Information on the safety of this plant was searched for in the Complete German Commission E Monographs compiled by the American Botanical Council and Global Information Hub on Integrated Medicine (GLOBinMED), Natural standard monograph, and Natural Medicines, comprehensive database, Google Scholar, Pubmed. Also, the safety of the active constituent of the plant was also searched. The pertinent journals were collected and the results tabulated as grade for indications of use, level for safety of consumption during pregnancy and safety of consumption during lactation. The level of evidence for therapeutic use and evidence for harm were evaluated based on ‘Grades for evidence for efficacy’ and levels for evidence for harm as displayed in Tables 1 and 2 respectively [13-16].

GRADE	EVIDENCE
A	VERY STRONG SCIENTIFIC EVIDENCE Statistically significant evidence of benefit from one or more systemic reviews/meta-analysis
B1	STRONG EVIDENCE Statistically significant evidence of benefit from one or more properly conducted random control trials (RCTs).
B2	GOOD SCIENTIFIC EVIDENCE Statistically significant evidence of benefit from one or more RCTs. The RCTs, however, are either of small sample size OR have discrepancies in their methodologies
C	FAIR SCIENTIFIC EVIDENCE Statistically significant evidence of benefit from one or more cohort studies OR outcome studies.
D	WEAK SCIENTIFIC EVIDENCE Evidence from case series
E	INDIRECT AND/OR CLINICAL EVIDENCE Evidence from case reports OR expert opinion OR laboratory studies
F	HISTORICAL OR TRADITIONAL EVIDENCE Historical or traditional use by medical professionals, herbalists, scientists, or aboriginal groups.

Table 1: Grades for the evidence for efficacy.

LEVEL	EVIDENCE
1a	STRONG SCIENTIFIC EVIDENCE Statistically significant evidence from one or more systematic reviews or RCTs.
1b	GOOD SCIENTIFIC EVIDENCE Statistically significant evidence from one or more cohort studies OR control study.
1c	WEAK SCIENTIFIC EVIDENCE Evidence from one or more case series.
2	VERY WEAK SCIENTIFIC EVIDENCE Evidence based on case reports.
3	IN VITRO SCIENTIFIC EVIDENCE Evidence based on scientific studies conducted on animals, insects or microorganisms OR laboratory studies on human cells.
4	INDIRECT EVIDENCE Evidence based on scientific theory OR expert opinion.
5	UNKNOWN No available information.

Table 2: Level for evidence for harm.

Results

INDICATION	GRADE
Anti-inflammatory, antipyretic and analgesic [17-19]	E
Antimalaria [11,12,20,21]	E
Antifungal [8,22,23]	E
Antibacterial [12,24-26]	E
Antibacterial [27]	B1
Antiviral [12]	E
Hepatoprotective [9,10,28]	E
Antioxidant [9,29-31]	E
Immunostimulant [32]	E
Hypoglycaemia [5,33]	E
Antiulcer [34,35]	B1
Antifertility [36]	C
Antinephrotoxic [37]	E
Spermicidal [38]	E
Antitumor [34]	E
Contraceptive [39,40]	C
Antitrypanosomal [34]	F
Anthelminthic [41]	E
Psoriasis [42,43]	B1
Anti-HIV/AIDS [44,45]	E
Anticholesterol and antihypertensive effects [46,47]	E
Antinoiceptive effect [48,49]	E
Neuroprotective [50]	E
Antiplaque [51]	C

Table 3: Indications for use.

Acute toxicity

Toxicity studies of different parts of *Azadirachta indica* extracts and purified compounds, have been investigated in different animal models such as rats, mice, chicken, monkey [8]. In rodents, acute toxicity studies of the neem seed oil, showed the 24 hr LD_{50} to be 14 ml/kg bw in rats and 24 ml/kg bw in rabbits [52].

Intraperitoneal administration of *Azadiractha indica* (1000 mg kg^{-1} of extract) did not result in any sign of toxicity in rats, nor did it alter body or organ weight during a three week administration. However, oral administration of 3.200 mg kg-1 resulted in 100% mortality in rats [34].

Evaluation of neem-based pesticides for safety, showed that the non-aqueous extracts appear to be the most toxic with an estimated safe dose (ESD) of 0.002 and 12.51 g/kg bw, as compared to the aqueous extracts and the unprocessed seed oil which are less toxic and have ESD of 0.26-0.3 mg/kg bw/day, 2µl/kg bw/day respectively [53]. The authors also reported that most of the pure compounds isolated from AI, for example, Azadirachtin have a low toxicity with ESD of 15mg/kg bw/day. Also, sub-acute or chronic exposure of extracts prepared from AI, produced a reversible effect on the reproduction of both male and female mammals tested. Similar finding was reported by Raizada and Srivastava (2007), [54] on azadirachtin. No pharmacotoxic or teratogenic signs, mortality, changes in weight or changes in blood tests was observed when azdirachtin, was administered by mouth to male and pregnant female rats up to 1500 mg kg^{-1} day^{-1} for 90 days. These researchers suggested that the dose, 1500 mg kg-1 as basal to determine at which level no effects were caused by the compound and thus accepted as a safety margin. No toxic effect was reported in two generations, of male and female rats treated with Neem oil at 5, 25, and 50 mg kg^{-1} added to the feed and ad libitum [54].

The LD_{50} values of neem oil were found to be 31.95 g/kg [55]. The acute toxicity study of neem leaf aqueous extract in chicken showed an intraperitoneal LD_{50} of 4800 mg/kg, and clinical signs were dose dependent [56].

The lethal median doses (LD_{50}) reported for neem leaf and stem bark extracts were 31.62 and 489.90 mg/kg body weight, respectively [21]. While the LD_{50} of water extract of *Azadirachta indica* leaves and seeds were 6.2, 9.4 mL kg^{-1}, respectively [57]. Using probit analysis, the LD_{50} and LD_{90} values were reported to be 8.4 and 169.8 µg/fly of neem extract, respectively [58]. A study of acute oral toxicity in mice showed an LD50 value of about 13 g/kg body weight [59].

	GRADE
Antifertility effect [8,60-64]	E

Table 4: Effect on Male and Female Reproductive System.

An increase in the frequency of spermatozoa with abnormal head morphology, chromosome strand breakages or spindle distribution and a reduced sperm count were observed, following oral administration of a crude ethanolic extract of neem leaves to adult male mice for 6 weeks at a rate of 0.5, 1.0 and 2.0 g/kg bw [65]. Similar effects were reported in rats [66].

When neem oil at 5, 25, and 50 mg kg^{-1} was administered through the feed and ad libitum to male and female rats, no toxic effect was reported in two generations, of treated rats (Raizada and Srivastava [54].

	Level
Abortion [39,67]	3
Reduction of follicles [68]	3
Antifertility in females [32,69]	3
Increase in PCV, WBC, Hb, RBC and Pl count [1]	3
Congenital deformation [70,34]	3
Teratogenic [71,72]	3

Table 5: Safety of consumption during pregnancy.

Neem extract given to rodents and primates at early post-implantation stage terminated the pregnancy, with no residual permanent effect. Fertility was regained in subsequent cycles [39,40]. Neem extract has fewer side effects as compared to

steroidal contraceptives. This is due to lack of estrogenic, antiestrogenic or progesteronal properties [73].

A purified fraction of neem (*Azadirachta indica*) seeds, given orally in rats caused a specific activation of T lymphocyte cells of CD8+ subtype as well as phagocytic cells followed by elevation in cytokines gamma-interferon and TNF typical of early post implantation contraceptive effects [67]. Unlike azadirachtin, Neem seed oil, caused congenital malformations on gestation in rats treated with 1.2 mL of Neem seed oil (G1), and on another group treated orally with 1 mg mL-1 of an azadirachtin based product (G2) [70].

	Level
Postnatal development and reproductive performance [74]	3
Improvement in milk production [75]	3
No decrease in body weight [34].	3

Table 6: Safety of consumption during lactation.

Neem ethanol extract was found safe at doses 65, 135 and 200 mg/kg, when administered to female wister rats during pregnancy and lactation. No signs of systemic or reproductive toxicity, such as piloerection, weight loss, diarrhea, stereotypes, vaginal bleeding, ataxia, coma, or death in the parents treated with the extract on the 4th, 5th, and 6th day of gestation, and from the 1st to the 15th day postpartum. The authors demonstrated through their findings, that the lactating rats and their offspring did not show clinical signs compatible with systemic toxicity, and no evidence of any significant decrease in body mass, no stillborn or any neonatal malformation [74].

Parts Used

Bark, seed, leaves, fruit, seed oil.

Chemistry

Nimbidin, sodium nimidate, nimbin, nimbitin, nimbidol, nimbolinin, nimbolide, gedunin, azadirachtin, mahmoodin, azadirone, amoorastatin, vepinin and vilasinin gallic acid, (-) epicatechin, catechin, margolone, margolonone, isomargolone, cyclic trisulphide, cyclic tetrasulphide, polysaccharides, polysaccharides G1a, G1b, polysaccharides GIIa, GIIIa, NB-II peptidoglycan, meliacin, meliatetraolenone, sesquiterpene, 2,6-Bis-(1,1)-dimethylethyl-4-methyl phenol, azadirone, benzopyranoids, prenylated flavones, fatty acid esters, salannin, cardenolide, nimocinol, propyldisulfide, quercetin (a flavonoid) and nimbosterol (β-sitosterol), triterpenes. Other components of neem are sodium nimbinate. Other constituent of neem leaves include protein, carbohydrates, minerals, calcium, phosphorus, vitamin C, carotene, etc. Others include glutamic acid, tyrosine, aspartic acid, alanine, praline, glutamine and cystine like amino acids and several fatty acids (dodecanoic, tetradecanoic, elcosanic, etc.) [4,5,6,7,76].

Drug Interactions

Neem oil should be used with caution in insulin-dependent diabetic patients or patients taking oral antihyperglycaemic drugs as the oil may reduce blood sugar [77].

Pharmacology

Based on *invitro* studies,human spermatozoa treated with extracts of AI, decreased within 20 seconds with minimum effective spermicidal concentrations of 2.91 ± 0.699 [60,8]. The mechanism of action is by controlling the level of fructose and sperm parameters in rats' vas deferens [61].

From *invivo* studies, a significant decrease ($p<0.01$) in total testosterone, total bilirubin and K+ in serum was observed, in male wistar rats treated for 10 weeks with *Azadirachta indica* plant extracts [8].

A significant leukocytic infiltration in the uterine epithelium between days 3 and 5 post coitum, and an induction of a pre-implantation block in fertility was observed when fertile female wistar rats were treated with a single dose of neem oil via the intrauterine route, [32].

Unlike azadirachtin, neem seed oil caused congenital malformations during gestation in rats treated with 1.2 mL of neem seed oil (G1), while in another group treated orally with 1 mg mL-1 of an azadirachtin (G2), no such malformation was seen [70].

In a study using lactating rats to investigate the effect of AI on blood paramerters, such as the packed cell volume (PCV), hemoglobin concentration (Hb), red blood cell (RBC), white blood cell (WBC), and platelet (PLT) and blood glucose level, AI at concentrations of 400 mg/kg and 600 mg/kg significantly increased PCV and RBC and caused a decrease in blood glucose level in a dose dependent manner. In pregnant rats, *Azadirachta indica* at all doses investigated significantly increased all blood parameters (PCV, Hb, RBC, WBC, and platelets) measured. AI at all doses investigated also significantly decreased the blood glucose level. At doses of 200, 400, and 600 mg/kg body weight, extracts of AI, caused significant increases in non-pregnant rats, the PCV, HB, RBC, and WBC values compared with control values at all doses investigated while at 200 mg/Kg, *Azadirachta indica*, did not significantly increase the RBC value compared to control. The platelets were not significantly different among the various groups. The blood glucose level was significantly reduced at all doses of AI investigated when compared with the control [1].

Azadirachta indica has demonstrated antiulcerogenic effects in humans [35]. In albino rats, AI extract (100-800 mg/kg p.o., 100-25 mg/kg i.p.) significantly inhibited gastric ulceration induced by indomethacin (40 mg/kg) via histamine H(2) receptor [34].

Discussion

The different parts of *Azadirachta indica*, have been shown to possess some beneficial therapeutic effect. There is no strong clinical evidence supporting the use of AI in pregnancy or during lactation. Toxicity information on humans is scarce in the literature [8], except for allergic reaction of AI pollen in an Indian

Community [78]. Most of the toxicity information is derived from observation, *invitro* and animal studies [53].

Sub-acute or chronic exposure of extracts prepared from AI, caused a reversible effect on the reproduction of both male and female mammals tested. Notwithstanding this, the authors recommended that the use of extract of *Azadirachta indica*, should not be discouraged but used cautiously [53].

Although, no risk has been reported on the use of this plant through its traditional or common use, neem oil extract, was reported to cause vomiting, hepatic toxicity, metabolic acidosis, and encephalopathy in children, [79]. In another study on rat model, administration of leaf sap caused an antianxiety effect at low doses, whereas such effects were not observed at high doses [80]. Azadirachtin a pure compound from neem plant did not show toxicity even at 5 g/kg bw on rats model [81].

A more rigorous and well controlled- clinical research is needed before this plant can be used during pregnancy and lactation. It is important for consumers and clinicians to know that AI has the potential to interact with some prescription medications such as antidiabetic drugs [77]. This is particularly important in the developing tropical countries where AI is used for the management of malaria.

Recommendation

Due to *Azadirachta indica's* potential to affect male and female fertility, caution should be exercised in the use in human by both gender. Male and female partners planning to start a family should avoid the use of AI extracts.

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