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Modulatory Effects of *in-utero Azadirachta indica* Leaf Meal on Hepatic Function, Lipid Profiles, Oxidative Balance, and Lipase Enzyme Dynamics in Wistar Rat Offspring

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ABSTRACT

Background and Objective: The absence of information on how prenatal exposure to *Azadirachta indica* leaf meal affects offspring's hepatic function, lipid metabolism, and oxidative balance is addressed in this study. This study investigated the impact of dried *Azadirachta indica* (AI) leaf powder on liver function, lipid metabolism, and oxidative status during pregnancy in the offspring of Wistar rats. **Materials and Methods:** Twelve male Wistar rats and 18 pregnant Wistar rats were used in the *in vivo* experimental design of the investigation. Throughout their gestation, pregnant rats were given either a regular diet or diets supplemented with different concentrations of dried leaf meal (DLM) from *Azadirachta indica*. Offspring were evaluated postnatally for oxidative balance, lipase activity, lipid profiles, and hepatic function. They were divided into four groups: Treated males (TM), treated females (TF), control males (CM), and control females (CF). Biochemical assays were used to analyse the tissues of the liver and blood. The ANOVA and Tukey's *post-hoc* tests were used to analyse the data, and $p<0.05$ was considered statistically significant. **Results:** The TM and TF exhibited significantly decreased levels ($p<0.05$) of liver enzymes, Triglycerides (TG), and Low-Density Lipoprotein (LDL), along with significantly increased levels ($p<0.05$) of High-Density Lipoprotein (HDL), liver lipase (HL), and lipoprotein lipase (LPL) when compared to controls. Additionally, superoxide dismutase (SOD), reduced glutathione (GSH), and catalase (CAT) levels significantly increased in TM and TF, while the lipid peroxidation marker malonaldehyde (MDA) significantly decreased in these groups compared to controls. **Conclusion:** Overall, this study offers promising evidence that *Azadirachta indica* supplementation during pregnancy may benefit the health of offspring, particularly concerning liver function and lipid regulation. These findings encourage future research exploring the potential of unique compounds like *Azadirachta indica* in supporting maternal and foetal health and preventing future metabolic disorders.

KEYWORDS

Azadirachta indica, hepatic, lipase, oxidative, sprague-dawley

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INTRODUCTION

The history of metabolic disorders has been traced to the addition of prenatal lifestyle manipulations and nutritional insults[1]. The hypothesis on fetal developmental programming and chronic diseases is not novel; however, it has given rise to a persistent interest in the impact of gestational environment on foetal/neonatal development and growth[2]. Foetal developmental programming as a law infers that the foetal environment during its developmental phase plays a formative role in determining the risk of disease during the stages of postnatal life[3]. There is enough evidence in the literature that describes an advantageous relationship between maximum in-utero nutritional exposure and development at the postnatal stage, including the elevated risk to diseases in the presence of an insult to the maternal environment[4,5].

Azadirachta indica, commonly known as neem or Indian lilac, is a versatile plant with a long history of use in traditional medical systems, especially Ayurveda[6]. Neem is known for various pharmacological properties such as anti-inflammatory, antibacterial, antioxidant, and hepatoprotective properties[7,8]. These medicinal properties are believed to be due to the wide variety of bioactive compounds present in neem leaves, seeds, bark, and other parts of the plant. Neem contains a wide range of bioactive compounds, some of which have been extensively studied for their potential health benefits. Neem leaves also contain various polyphenols that contribute to their antioxidant and anti-inflammatory properties. These polyphenols, including gallic acid and catechins, are being investigated for potential therapeutic uses[6]. Neem is a source of terpenoids such as limonoids and azadirachtin. Limonoids have shown hepatoprotective properties in animal studies by enhancing liver detoxification enzymes and reducing liver damage[8]. Neem's hepatoprotective effects have been the subject of scientific research interest. Studies conducted in animal models have shown that neem extract can protect the liver from various forms of damage, including chemical-induced hepatotoxicity[7,8]. It is believed that neem's hepatoprotective properties involve lowering oxidative stress, regulating liver enzymes, and blocking inflammatory pathways[9]. According to Heyman *et al*.[10] neem's antioxidant qualities are well-established, and oxidative stress arises when the body's capacity to neutralise reactive oxygen species (ROS) with antioxidants is out of balance with the creation of ROS[11]. Oxidative stress is associated with many health conditions, including liver disease[12].

The prevalence of metabolic disorders on a global scale is increasing, and the potential therapeutic benefits of AI are becoming increasingly apparent. However, there is a considerable lack of knowledge regarding the potential impact of *in-utero* AI dry leaf meal on liver function, lipid profiles, and oxidative equilibrium in the progeny. This gap must be bridged in order to gain a better understanding of the potential advantages of AI as a prenatal dietary intervention and its implications for the prevention of metabolic disorders in the following generations. This study aims to investigate the modulatory effects of prenatal (in-utero) administration of *Azadirachta indica* leaf meal on the hepatic function, lipid profiles, oxidative balance, and lipase enzyme dynamics in Wistar rat offspring.

MATERIALS AND METHODS

Study area: The study was carried out at the Animal House of the Department of Science Laboratory Technology, D.S Adegbenro ICT Polytechnic, Itori, Ogun State, Nigeria between April to August, 2022.

Experimental animals: Eighteen female Wistar rats with a weight range of 140-180 g were used and were sheltered in cages with quality lighting conditions, 12 hrs light. They had free access to tap water with quality food and were acclimatized for 1 week.

Ethical clearance: The experimental protocols were in accordance with the regulations of the Experimentation Ethics Committee on Animals Use of the College of Medicine of the University of Lagos, Lagos State and in accordance with the United States National Academy of Sciences Guide for the Care and Use of Laboratory Animals.

Table 1: Proximate composition of dried AI leaf

Feed contents	Percentage
Moisture	12.36
Fat	2.21
Crude protein	15.84
Carbohydrates (CHO)	51.63
Crude fibre	13.13
Ash	4.83

Neem leaf (*Azadirachta indica*) AI collection: Fresh matured AI leaves were harvested from an *A. indica* tree in a village, Afowowa in Ewekoro local government area of Ogun State, Nigeria, and were identified at the Biology unit of D.S. Adegbenro ICT polytechnic, Eruku-Itori, Ewekoro, Ogun State. The sample identified was deposited in the herbarium of D.S Adegbenro ICT Polytechnic with a voucher number DSA4756 and the collected samples were air dried and ground with a blender to obtain the powdered form. Ethical approval was sought and given to conduct this investigation by the Institutional Research and Ethics Committee with ethical code DSAERC12-2022.

Composition of experimental diet: Approximately 750 g of powdered AI leaf was pelleted with 25 kg of standard rat chow to constitute the AI dry leaf meal (DLM) and 50 kg of another regular rat chow to constitute the control diet (CONT) Table 1.

Mating and grouping: Female Wistar rats were subjected to sexual coitus with approved male breeders overnight, one male per two females, and they were maintained on their respective diet throughout gestation. The day on which spermatozoa was observed on a vaginal smear that was washed with normal saline NaCl 0.9% was assigned as conception day 0[13]. The rats were thereafter allocated to 1 of the 4 groups to be treated with either a control diet or *A. indica* dried leaf meal. Water and food were made available for all rats and grouped thus (6 animals per group):

- **Group A:** Control male (CM) (treated with CONT diet throughout the experiment)
- **Group B:** Control female (CF) (treated with CONT diet throughout the experiment)
- **Group C:** Treated male (TM) (treated with *A. indica* dried leaf meal only during gestation)
- **Group D:** Treated male (TM) (treated with *A. indica* dried leaf meal only during gestation)

All the pups were transferred CONT diet, excluding the CM and CF, until the end of the procedure, postnatal day (PND) 49. The pups were reduced to 8-10 on postnatal PND 1 (birth, day 0), weaned on PND 21, and housed in groups of 3 or 4, male and female offspring separately per cage.

Isolation of tissue: The rats were sacrificed on PND 49 through dislocation of the cervical vertebral following a light anesthesia, and were dissected livers were dissected, washed in ice water, and rinsed with 1.15% KCl, which was blotted and weighed[14-16].

Blood sample: About four (4 mL) of blood sample was obtained via cardiac puncture into plain bottles and was centrifuged at 3000 rpm for 15 min, with the serum carefully separated with a rubber pipette in a clean Eppendorf bottle and stored at -20°C until analyses[17-19].

Oxidative stress study: All parameters of oxidative stress were determined thus; superoxide dismutase (SOD) and reduced glutathione (GSH)[20], catalase and lipid peroxidation's index, malonaldehyde (MDA)[21].

Liver function assay: Albumin, Alkaline Phosphatase (ALP), Alkaline Amino Transferase (ALT), and Aspartate Amino Transferase (AST) were assayed using serum samples with the aid of an automated analyzer (Mindray BS-120, Chema Diagnostica, Italy).

Determination of lipid profile: Cholesterol, triglyceride, high-density, and low-density lipoprotein levels were assayed from the liver homogenate samples with the aid of an automated Analyzer (Mindray BS-120, Chema Diagnostica, Italy).

Quantification of castelli index: According to Igbayiloa *et al.*[14] the Castelli indices I and II were determined using the following equations:

$$\text{Castelli index I} = \frac{\text{Cholesterol}}{\text{HDL cholesterol}}$$

$$\text{Castelli index I} = \frac{\text{LDL}}{\text{HDL cholesterol}}$$

Determination of hepatic lipase (HL) and Lipoprotein Lipase (LPL): Hepatic lipase (HL) and Lipoprotein Lipase (LPL) activity were determined in liver tissue homogenate[22].

Statistical analysis: Data were presented as the mean standard error of mean (SEM), and GraphPad Prism Software (Graph Pad, Inc., La Jolla, CA, USA) was used for statistical analyses. One-way Analysis of Variance (ANOVA) with *post hoc* Tukey's multiple comparison test was used. The level of significance was set at ($p=0.05$).

Table 1 revealed the percentage (%) proximate composition of the dried AI leaf and the constituents include: Moisture (12.36), fat (2.21), crude protein contents (15.84), carbohydrates (51.63), crude fibre (13.13), and ash (4.83).

RESULTS AND DISCUSSION

Mineral compositions of dried AI leaf: Results presented in Tables 2 and 3 revealed the presence of mineral composition in dried AI leaf.

The nutritional analysis of dried *A. indica* leaf revealed a rich profile of vitamins and minerals. Among the vitamins, vitamin C was found in the highest concentration (308.542 mg/100 g), followed by vitamin B6 (35.611 mg/100 g) and vitamin E (3.289 mg/100 g). Lower concentrations were recorded for vitamins A (1.864 mg/100 g), B1 (0.312 mg/100 g), B2 (0.343 mg/100 g), B9 (2.864 mg/100 g), and D (1.681 mg/100 g) in Table 2.

Mineral analysis showed that calcium (1569.712 mg/100 g), sodium (1389.167 mg/100 g), and potassium (1216.738 mg/100 g) were the most abundant macrominerals. Other notable minerals included magnesium (1096.443 mg/100 g), phosphorus (628.136 mg/100 g), zinc (4.386 mg/100 g), and iron (3.412 mg/100 g), while lead was present in trace amounts (0.121 mg/100 g), suggesting the need for toxicological assessment in Table 3.

Outcome of DLM of *A. indica* on markers of hepatic function (AST, ALT, and ALP): Figure 1 shows a statistically significant reduction in aspartate aminotransferase (AST) levels ($p<0.05$) for both the TM and TF groups relative to the CM and CF controls, suggesting a potential hepatoprotective effect of the treatment in minimizing hepatic cellular damage. On the other hand, Fig. 2 indicates that no significant shift in the activity of Alanine Aminotransferase (ALT) ($p>0.05$) was observed between the same groups, indicating that the therapy is capable of selective effects on some liver enzymes, or ALT activity is insensitive to hepatic modulation. Figure 3 illustrates a significant decrease in Alkaline Phosphatase (ALP) levels ($p<0.05$) in TM and TF, compared to CM and CF, indicating possible improvement of biliary function or relief of cholestatic stress. Of greater significance, ALP levels in TM were significantly greater than in TF ($p<0.05$), suggesting a sex difference or treatment-related variation in the degree of hepatic response or enzyme control.

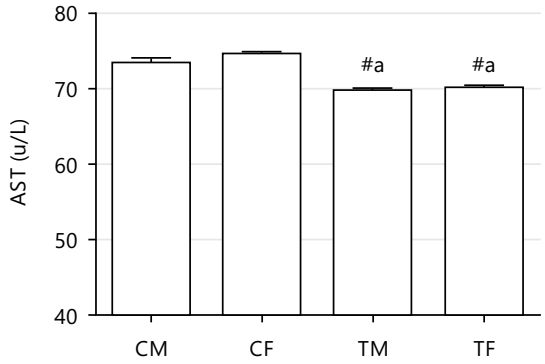

Fig. 1: Outcome of DLM of *A. indica* on AST in CONT and treated rats
Values represent Mean±SEM; n = 6. Significant levels ($^{\#}p<0.05$ vs CM, $^{a}p<0.05$ vs CF)

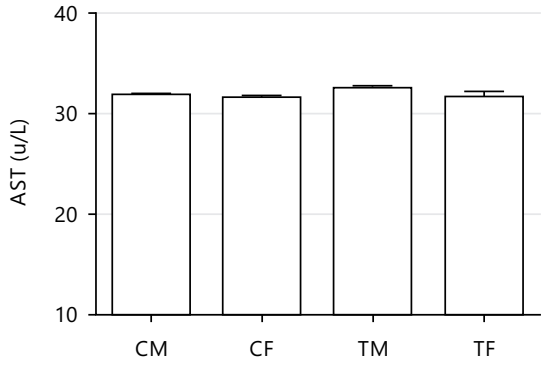

Fig. 2: Outcome of DLM of *A. indica* on ALT in CONT and treated rats
Values represent Mean±SEM; n = 6. Significant levels (#p<0.05 vs CM, $^{a}p<0.05$ vs CF)

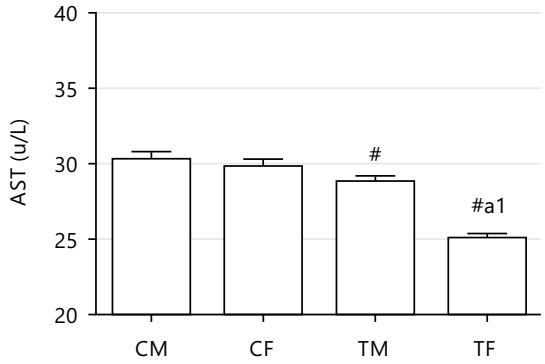

Fig. 3: Outcome of DLM of *A. indica* on ALP in CONT and treated rats
Values represent Mean±SEM; n = 6. Significant levels ($^{\#}p<0.05$ vs CM, $^{a}p<0.05$ vs CF)

Table 2: Vitamin analysis of dried AI leaf

Vitamins	Values (mg/100 g)
A	1.864
B1	0.312
B2	0.343
B6	35.611
B9	2.864
C	308.542
D	1.681
E	3.289

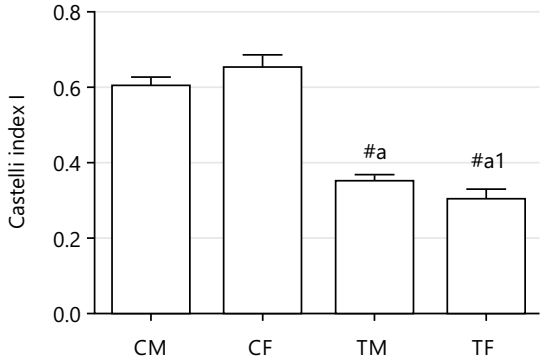

Fig. 4: Outcome of DLM of *A. indica* on Castelli index I in CONT and treated rats
Values represent Mean±SEM; n = 6. Significant levels ($^{\#}$p<0.05 vs CM, ^{a}p<0.05 vs CF)

Table 3: Mineral compositions of dried AI leaf

Minerals	Values (mg/100 g)
Sodium (Na)	1389.167
Potassium (K)	1216.738
Calcium (Ca)	1569.712
Magnesium	1096.443
Zinc (Zn)	4.386
Potassium (P)	628.136
Iron (Fe)	3.412
Lead (Pb)	0.121

Table 4: Outcome of outcome of DLM of *A. indica* on CHOL, TG, HDL, LDL, HL and LPL in CONT and treated rats

Parameter	CM	CF	TM	TF
CHOL (mmoL/L)	1.90±0.05	2.70±0.07$^{\#}$	1.68±0.03$^{\#a}$	1.59±1.91$^{\#a1}$
TG (mmoL/L)	3.60±0.04	3.23±0.03	2.00±0.04$^{\#a}$	3.36±0.04$^{\#a1}$
HDL (mmoL/L)	0.92±0.37	0.92±0.37	1.20±0.03$^{\#}$	1.30±0.04$^{\#a}$
LDL (mmoL/L)	1.10±0.04	1.00±0.031	0.72±0.37$^{\#a}$	0.70±0.03$^{\#a}$
HL (unit/mg/min)	170.0±4.0	135.0±2.89$^{\#}$	190.0±7.07$^{\#a}$	218±2.00$^{\#a1}$
LPL (unit/mg/min)	59.0±2.92	66.0±2.45	75.0±1.58$^{\#}$	107.00±5.6$^{\#a1}$

Values represent Mean±SEM; n = 6, Significant levels ($^{\#}$p<0.05 vs CM, ^{a}p<0.05 vs CF), CHOL: Total cholesterol, TG: Triglycerides, HDL: High-density lipoprotein cholesterol, LDL: Low-density lipoprotein cholesterol, HL: Hepatic lipase activity, LPL: Lipoprotein lipase activity, Values are expressed as mean±standard deviation, CM: Control male, CF: Control female, TM: Treated male and TF: Treated female

Outcome of DLM of *A. indica* on indices of lipid homeostasis: Table 4 reveals a statistically significant reduction (p<0.05) in the levels of total cholesterol (CHOL) in the TM and TF groups compared to CM and CF controls, which suggests that the treatment lowers cholesterol levels. Triglyceride (TG) levels were also significantly lower (p<0.05) in TM and TF compared to CM, indicating improved lipid metabolism. However, the TG level was significantly greater (p<0.05) in TF compared to CF and TM and may represent a sex-dependent action of the treatment or a difference in lipid mobilization.

The level of HDL was increased dramatically (p<0.05) in TM and TF relative to CM and CF, indicating a likely cardioprotective action of the treatment. The level of HDL, however, was significantly reduced (p<0.05) in TM relative to TF, indicating sex-specific differences in the regulation or sensitivity of HDL. The LDL concentrations were higher (p<0.05) in TM and TF than in CM and CF, possibly raising atherogenic concern, although LDL was lower (p<0.05) in TM than in TF, again suggesting sex-dependent modulation.

Hepatic lipase (HL) activity was higher (p<0.05) in TM and TF than in CM and CF, indicating augmented hepatic lipoprotein remodeling. However, HL activity was also notably decreased (p<0.05) in TM than TF, which might represent gender-specific variations in the hepatic handling of lipids. In like manner, activity

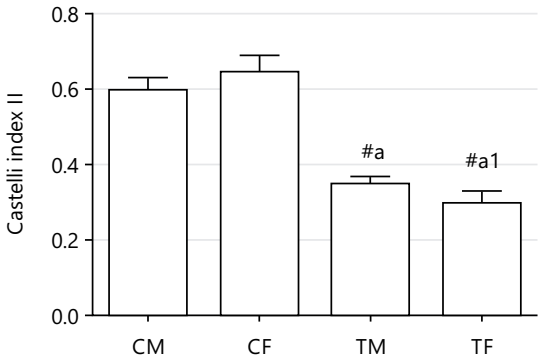

Fig. 5: Outcome of DLM of *A. indica* on Castelli index II in CONT and treated rats
Values represent Mean±SEM; n = 6. Significant levels ($^{\#}p<0.05$ vs CM, $^{a}p<0.05$ vs CF)

Table 5: Outcome of DLM of *A. indica* on GSH, SOD, CAT and MDA in CONT and treated rats

Parameter	CM	CF	TM	TF
GSH (µmoL/mL/mgpro)	79.71±2.04	83.34±0.44$^{\#}$	121.2±2.41$^{\#a}$	96.42±0.69$^{\#a1}$
SOD (µmoL/mL/mgpro)	0.77±0.01	0.83±0.02$^{\#}$	0.84±0.01$^{\#a}$	0.92±0.01$^{\#a1}$
CAT (µmoL/mL/mgpro)	2.47±0.23	3.08±0.24	4.11±0.23$^{\#a}$	4.34±0.15$^{\#a}$
MDA (pmoL per mg of protein)	5.95±0.27	3.74±0.22$^{\#a}$	5.11±0.05$^{\#a}$	5.20±0.01$^{\#a}$

Values represent Mean±SEM; n = 6, Significant levels ($^{\#}p<0.05$ vs CM, $^{a}p<0.05$ vs CF), GSH: Reduced glutathione, SOD: Superoxide dismutase, CAT: Catalase, MDA: Malondialdehyde, Values are expressed as Mean±Standard Deviation, CM: Control male, CF: Control female, TM: Treated male, TF: Treated female

for the lipolytic enzyme LPL was elevated markedly ($p<0.05$) in TM and TF in contrast to CM and CF, representing better clearing of peripheral triglycerides, and depressed considerably ($p<0.05$) in TM than in TF, indicating an additive enzyme response to differences between the genders.

Figure 4 indicates a significant rise ($p<0.05$) in Castelli index I (TC/HDL-C) in TM and TF over CM and CF, which may indicate a less favorable lipid profile with increasing HDL. Interestingly, this index was significantly lower ($p<0.05$) in TM than in TF, reflecting an improved lipid balance in treated males. Figure 5 also shows an increase ($p<0.05$) in Castelli index II (LDL/HDL-C) in TM and TF compared to CM and CF, with a significant decrease ($p<0.05$) in TM compared to TF, once more demonstrating sex-related differences in lipid ratio responses.

Generally, these results demonstrate that the treatment transforms lipid metabolism by reducing CHOL and TG levels and enhancing HDL, LPL, and HL activity, but also raises LDL and Castelli indices in female rats. This dual action underscores the need to investigate the long-term cardiovascular impact of the treatment as well as its sex-related metabolic pathway.

Outcome of DLM of *A. indica* on markers of oxidative stress (GSH, SOD, CAT and MDA): Table 5 indicates a significant upregulation ($p<0.05$) of glutathione (GSH) activity in TM and TF groups compared with CM and CF, indicating enhanced intracellular antioxidant capacity and potential relief of oxidative stress by treatment. Of particular note, GSH activity was significantly higher in TM compared with TF ($p<0.05$), suggesting sex-dependent differential enhancement of redox homeostasis, with males responding more strongly. Superoxide dismutase (SOD) activity also increased significantly ($p<0.05$) in TM and TF relative to CM and CF, reflecting increased enzymatic neutralization of superoxide radicals. The SOD activity was significantly lower in TM than in TF ($p<0.05$), and this suggests that the female cohort may have had a more intense activation of this specific antioxidant pathway. The CAT activity also showed the same pattern with steep increases ($p<0.05$) in TM and TF compared to controls, indicating increased hydrogen peroxide decomposition and increased oxidative defense. Of interest was the fact that CAT activity was also significantly ($p<0.05$) lower in TM compared to TF, corroborating the observation of more vigorous enzymatic antioxidant activity in females. Furthermore, the table shows a significant

reduction ($p<0.05$) in an unspecified parameter (most likely an indicator of oxidative damage such as MDA) in TM and TF compared to CM, which shows reduced lipid peroxidation following treatment. Conversely, a significant increase ($p<0.05$) was observed in TM and TF compared to CF, which could mean that as far as CF is concerned, although TM and TF are superior to CM, there is some residual oxidative stress. Collectively, these findings indicate that the treatment is effective in upregulating the endogenous antioxidant defense system by enhancing the activities of GSH, SOD, and CAT. The sex differences observed are reflective of a more robust antioxidant enzyme response in females (SOD and CAT) and a more robust GSH response in males, implications for the sex-specific metabolism-driven tailoring of antioxidant therapies.

The use of AI dried leaf meal as a dietary supplement during pregnancy has raised interesting questions about its effects on liver function, lipid homeostasis, and oxidative balance in Wistar rat offspring. The AI is one of those plants which has been reported to possess chemoprotective potential and a highly effective antioxidant effect[23-25]. Results from the present investigation revealed the hepatoprotective effect in rat offspring exposed to *A. indica* dried leaf meal, as the markers of liver function, such as AST, ALT and ALP reduced significantly and this strongly agrees with a previous study, which confirmed non-toxic effect of neem leaves extract at varying doses on rat liver[26]. One of the most important findings from studies of artificial feeding in mothers is that it can positively impact liver function in children. The liver plays a central role in metabolism and the regulation of various biochemical processes, making it a critical organ for overall health. Studies have shown that taking AI supplements by mothers may improve their children's liver function and reduce the risk of future liver disease. A study by Seriana *et al.*[24] investigated the effects of maternal AI supplementation on liver function in Wistar rat offspring. Researchers showed that the group exposed to AI during pregnancy had lower levels of liver enzymes such as ALT and AST and improved liver function. These results suggest that AI may have heptoprotective effects and have the potential for dietary interventions to support liver health in offspring. Maintenance of lipid homeostasis is essential for overall health and is closely associated with the development of metabolic disorders such as obesity and cardiovascular disease.

Interestingly, serum levels of TG, cholesterol, and LDL were decreased and HDL was increased in the offspring of rats exposed to dry leaf powder of *Azadirachta indica*. In this study, the mechanism by which *Azadirachta indica* dried leaf powder induced a decrease in lipid parameters was through stimulation of lipolysis and this is an accordance with the previous studies[27-29] and fatty acid utilization and/or inhibition of fatty acid synthesis in the liver of rodents[30]. Serum cholesterol levels in rat offspring were found to gradually decrease with increasing dietary intake of *A. indica* dried leaf powder. Although this pronounced effect probably indicates a complete reduction in lipid mobilization, *A. indica* dry leaf powder has an indirect inhibitory effect on the levels of HMG-CoA reductase, a key enzyme in the biosynthesis of cholesterol metabolism. A study by Chen *et al.*[30] investigated the effects of maternal AI supplementation on lipid homeostasis in Wistar rat offspring. The results showed that AI-exposed offspring had lower levels of serum triglycerides and total cholesterol compared to the control group. These results suggest that AI may play a role in preventing dyslipidemia, an important risk factor for metabolic syndrome.

The potential impact of *in utero* exposure to *Azadirachta indic*a dry leaf powder on Castelli index 1 and 2, which are established markers of cardiovascular risk, is an interesting area of research. These indices, calculated from lipid profile components, play an important role in assessing atherogenic risk and predicting cardiovascular disease outcomes. Studies on maternal nutritional supplementation with AI dry leaf powder and its effects on Castelli index 1 and 2 are still limited in the existing literature. However, AIs have been studied for their potential to modulate lipid metabolism, including their effects on total cholesterol levels, Low-Density Lipoprotein Cholesterol (LDL-C) levels, and High-Density Lipoprotein cholesterol (HDL-C) levels[31]. Studies with other diets and compounds have shown that a mother's diet can influence her child's lipid profile and thus cardiovascular risk. Therefore,

it is conceivable that *in utero* exposure to AIs may have similar effects on lipid metabolism and influence Castelli indices 1 and 2. The present study explored the role of AI dried leaf meal on Castelli indices 1 and 2, and the results were promising as the two indices significantly reduced in male and female offspring exposed to the treatment. Furthermore, hepatic lipase is an enzyme that has been severally investigated to be responsible for converting triglycerides into free fatty acids and glycerol, the acetyl CoA is produced when free fatty acids are broken down in the hepatic tissue, and the heightened scale of acetyl CoA leads to cholesterol, triglyceride and ketone bodies as a result of ketosis in the liver, an increase in the flow of free fatty acids promotes the production of very low density lipoprotein particles, which in turn are transformed to low density lipoprotein[32]. Hence, increased hepatic lipase activity in this study may not be unconnected with decreased triglyceride levels observed in offspring of rats exposed to perinatal neem leaf supplementation. Oxidative stress, characterized by an imbalance between reactive oxygen species (ROS) and antioxidants, is associated with various pathological conditions, including metabolic disorders. Maternal AI supplements have been studied for their potential to modulate oxidative balance in offspring[32]. Results showed that offspring exposed to AI had higher levels of antioxidant enzymes such as superoxide dismutase (SOD) and catalase, lower levels of lipid peroxidation products, and reduced oxidative stress. Antioxidant assay results evidenced by increased activity of markers of oxidative balance such as SOD, GSH, and CAT and concomitant decrease in MDA lipid peroxidation index in offspring of rats fed and treated with AI leaf-enriched diet showed an increase in oxidative balance. Lipid peroxidation is a well-known process of cell damage and is a chain reaction caused by free radicals. It is an indicator of oxidative stress in cells and tissues. Biswas *et al.*[7] investigated the antioxidant activity of neem leaf extract in rats exposed to oxidative stress. In this study, we found that neem supplementation significantly reduced lipid peroxidation markers, suggesting that it may play a protective role against lipid oxidative damage. Furthermore, oxidative stress during pregnancy can have long-term effects through epigenetic changes that influence susceptibility to diseases later in life[28]. In particular, the developing foetal liver is susceptible to oxidative damage, so interventions that reduce lipid peroxidation during pregnancy represent an attractive option to improve long-term health outcomes in the offspring.

CONCLUSION

This study demonstrates that prenatal supplementation with dried *Azadirachta indica* leaf powder positively influences liver function, lipid metabolism, and oxidative status in the offspring of Wistar rats. Treated offspring exhibited improved hepatic enzyme profiles, enhanced antioxidant levels, and favorable lipid parameters compared to controls. These findings suggest that *Azadirachta indica* may hold potential as a natural dietary intervention during pregnancy to promote offspring metabolic health. Further studies are warranted to elucidate underlying mechanisms, establish safe dosages, and evaluate translational relevance to human populations.

SIGNIFICANCE STATEMENT

This study identified the beneficial effects of prenatal supplementation with dried *Azadirachta indica* leaf powder on liver function, lipid metabolism, and oxidative balance in offspring of Wistar rats, which could be beneficial for improving fetal metabolic health and preventing early-onset metabolic disorders. This study will assist researchers in uncovering critical areas of maternal dietary influence on offspring metabolic programming that have remained unexplored by many. Consequently, a new theory on the role of phytochemicals in prenatal metabolic conditioning may be developed.

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