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Modulatory Effects of *Phoenix dactylifera* Fruit Extract on Pregnancy Parameters and Placental Specific Parameters of a Rat Model of Preeclampsia

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Abstract

Preeclampsia is a prevalent issue among pregnant women; however, drug treatment options in mild and severe preeclampsia are limited. The use of natural chemical compounds from plants looked promising, hence the necessity for this study. The aim of this study is to analyze the modulatory effects of *Phoenix dactylifera* fruit extract on pregnancy parameters and placental specific parameters of a rat model of preeclampsia using standard analytical methods. A total of twenty-four (24) healthy albino rats (100 - 200 g) of both sexes were housed and co-habited with fertile male rats in a ratio of 2:1 (F: M). The experimental animals were divided into four groups containing six animals each (Four female rats and two males) as follow: group 1- Control (received normal feed and water), group 2- (induced preeclampsia by administering 50 mg/kg/day of N (ω)-nitro-L-arginine methyl ester (L-NAME) on gestation day 11), group 3 and 4 (were induced preeclampsia in addition to 20 mg/kg/day losartan between gestation day 12-20 and 100 mg/kg/day *Phoenix dactylifera* fruit extract between gestation 5-18). The pilot study carried out ascertained that 100 mg/kg/day of *Phoenix dactylifera* fruit extract was the dose that gave the best treatment on the birth weight and number of live pups in rats. L-Name induced preeclampsia had a negative effect on the litter and maternal characteristics, placental antioxidants, cytokines and growth factors; however, treatment with losartan and *Phoenix dactylifera* fruit extract significantly reversed this effect. It can be concluded that *Phoenix dactylifera* fruit extract demonstrated positive modulatory effects on pregnancy parameters and placental specific parameters of preeclampsia induced rats, hence can be utilized as a potential agent for the management of hypertension and placental related complications during pregnancy.

Keywords: Preeclampsia; *Phoenix dactylifera* Fruit Extract; Placental Parameters; Litter Characteristics; Maternal Characteristics

1. Introduction

Preeclampsia disorder has plenty associated complications that affect both the mother and the unborn baby (Ożarowski et al., 2018). Assessment of the effects of plant phenolic compounds in animal models relevant to preeclampsia have been insufficiently reported, but the first studies which raise hope for new optional medicines for the treatment of preeclampsia have been published. The compounds most intensively investigated in this field are resveratrol, quercetin, curcumin, salvianolic acid A (danshensu), baicalin, epigallocatechin gallate, punicalagin, silibinin, and vitexin. Furthermore, plant extracts, as mixtures of various chemical compounds, were also investigated in preeclampsia. Eight promising plant extracts—*Euterpe oleracea* Mart., *Moringa oleifera* Lam., *Punica granatum* L., *Thymus schimperi* Ronniger, *Uncaria rhynchophylla* (Miq.) Miq. ex Havil., *Vitis vinifera* L.—were tested in animal models of preeclampsia (in all seven studies) (Batmomolin et al., 2020; da Silva et al., 2020; de Moura et al., 2007; Mergiaw et al., 2020; Nasifah,

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Soeharto & Nooryanto, 2017; Wu & Xiao, 2019), as well as *Brassica oleracea* L. and *Silybum marianum* (L.) Gaertner were investigated in two clinical trials concerning preeclampsia (one clinical trial in phase III is in progress) and though results look promising, more studies is required to combat preeclampsia. These studies are very much needed because drug treatment options in mild and severe preeclampsia are limited. Currently, only methyldopa is used as the first-line treatment of this disease (Ożarowski et al., 2018); therefore, *Phoenix dactylifera* (date plant) can be explored due to its pharmacological benefits (Al-Shwyeh, 2019). Findings from this study can provide safer alternative to this issue that has lingered for long and create room for new pharmacological possibilities.

2. Methodology

2.1. Plant collection and identification

Fresh *Phoenix dactylifera* (dabino) fruits were obtained from Kaduna state in the Northern part of Nigeria. The procured plant nuts were identified in the Department of Plant Science and Biotechnology, Imo State University, Owerri.

2.2. Sample preparation

Exactly 2 kg of *Phoenix dactylifera* fruits was washed, sorted, macerated and oven-dried at 40-50^{o}C to steady weight. This process produced dry red pomegranates as many as ±100 g. Furthermore, the dried fruits were blended until smooth, then dried pomegranate powder was soaked by using 900 ml of ethanol solvent (3 × 24 h) and evaporation process was carried out. The final result of evaporation process was the total extracts. In this study, total of 44 g of pomegranate extracts were obtained from 100 g of dried pomegranates in the form of essential oils (Ambarwati et al., 2017).

2.3. Experimental design

A total of twenty-four (24) healthy albino rats (100 - 200 g) of both sexes were utilized for this study. Albino rats were purchased from Mr Samuel of the Anatomy Department of Imo State University and carefully transferred to Animal house of Department of Biochemistry, Imo State University Owerri where the experimental animals were housed at room temperature controlled (27±3 ^{o}C) in cages with a 12; 12 light dark cycle and wire lid to allow proper ventilation for the whole period of the experiments. Albino rats were allowed access to standard animal chow and filtered water *ad libitum*. The female rats which were in estrous phase (identified by microscopic demonstration of typical epithelial cells on vaginal smear) were housed and co-habited with fertile male rats in a ratio of 2:1. (F: M). Four female rats and two males were house in a cage for the respective groups. The gestation day one was depicted as the day that copulation occurred as demonstrated by the presence of vaginal plugs and sperm cells on vaginal plug hence pregnancy, then male rats were separated from the female rats (Mergiaw et al., 2020).

The experimental animals were divided into four groups containing six animals each:

- Group I- Control (received normal feed and water)
- Group II- L-NAME (50 mg/kg/day) on gestation day 11 to induce preeclampsia
- Group III- preeclampsia + losartan drug (20 mg/kg/day) between gestation day 12-20
- Group IV- preeclampsia + extract (100 mg/kg/day) between gestation 5-18

2.4. Induction of preeclampsia (PE)

N (ω)-nitro-L-arginine methyl ester (L-NAME), an L-arginine analogue widely used inhibitor of nitric oxide synthase (NOS) activity both *in vitro* and *in vivo*, was used to induce PE in rats with oral doses of 50 mg/kg/d at gestation day 11 (Mergiaw et al., 2020). Losartan was used as a standard against preeclampsia in animals in Group III.

2.5. Calculation of LD_{50} for *Phoenix dactylifera* fruit Extract

The LD_{50} was calculated using the Organisation for Economic Co-operation and Development (OECD) (2001) model. This model also known as the "Up-and-Down Procedure" permits researchers to administer specified doses of extract to individual animals in a sequential manner. However, the dose to be given to the next rat should be adjusted based on the effect (fatality or safety) it has on the previous animal, before ascertaining the LD_{50} value using maximum likelihood estimation method. The LD_{50} is the dosage that caaused 50% fatality in animals.

LD_{50} was carried out using graded doses up to 5,000 mg/kg of *Phoenix dactylifera* fruit extract as shown in Tables 1A, 1B and 1C. Results of the acute toxicity produced no mortality in the rats. The treated rats also did not show signs of

severe toxicity like tremor, convulsions, writhing reflexes and agitations, but remained active and physically stable throughout the 24 hour period and a further 7 days of observation.

Table 1A Stage 1 Acute toxicity (LD_{50}) Evaluation of the *Phoenix dactylifera* fruit Extract in Rats

Group	Dose (mg/kg)	No. of Deaths	Percentage of mortality	Observations
1	10	0/3	0.00	No mortality observed, instead animals remained active and physically stable.
2	100	0/3	0.00	No mortality observed, instead animals remained active and physically stable
3	500	0/3	0.00	No mortality observed, instead animals remained active and physically stable

Table 1B Stage 2: Acute Toxicity (LD_{50}) Evaluation of the *Phoenix dactylifera* fruit Extract in Rats

Group	Dose (mg/kg)	No. of Deaths	Percentage of mortality	Observations
1	1000	0/3	0.00	No mortality observed, instead animals remained active and physically stable.
2	1600	0/3	0.00	No mortality observed, instead animals remained active and physically stable
3	3000	0/3	0.00	No mortality observed, instead animals remained active and physically stable

Table 1C Stage 3: Acute Toxicity (LD_{50}) Evaluation of the *Phoenix dactylifera* fruit Extract in Rats

Group	Dose (mg/kg)	No. of Deaths	Percentage of mortality	Observations
1	3500	0/3	0.00	No mortality observed, instead animals remained active and physically stable.
2	4000	0/3	0.00	No mortality observed, instead animals remained active and physically stable
3	5000	0/3	0.00	No mortality observed, instead animals remained active and physically stable

LD_{50} > 10,000 mg/kg body weight

3. Pilot study

Aim: To determine the optimum dose of *Phoenix dactylifera* extract needed to achieve an effect size of ≥10g increase in birth weight following an N (ω)-nitro-L-arginine methyl ester (L-NAME) induced preeclampsia.

Rationale: Preeclampsia is a leading cause of maternal death in Africa. Every year, more than 270,000 women die of preeclampsia in Africa, and Nigeria contributes to over 20% of this global burden of preeclampsia: the highest of any African country. Elevated maternal blood pressure, intrauterine growth restrictions, and low birth weight are the most worrisome pregnancy outcomes in preeclampsia. Antihypertensive drugs are usually administered to improve the maternal blood pressure; however, these treatments often do not improve the birth weight and in many reported cases, often causes fetal toxicity. Hence, the development of a therapy that can improve birthweight during preeclampsia is clear and imminent.

Design: Thirty rats was needed for this study for a power of $\alpha = \beta = 0.05$ at a 95% significance level. For mating purposes, 16-week-old inbred Wistar male rats weighing 180-200g with non-pregnant female rats of similar weight range were utilised in this study. Rats were provided suitable beddings and commercial rat feeds (UAC Nigeria Vital Feeds Grand Cereals, Jos, Nigeria) and water, *ad libitum*. The oestrous cycle of the rats was determined by vaginal lavage. In a ratio of 3:1 female to male, the rats were placed in separate cages and housed overnight together. Pregnancy was confirmed the next morning through a mating plug/positive vaginal smear check and recorded as gestation day 1. Pregnant rats were separated into individual metabolic cages and monitored until the study endpoint.

Preeclampsia was induced by daily intraperitoneal injection of 125mg/kg L-NAME on gestation day 12-15 as well as 0.3g/l L-NAME in drinking water, until endpoint. Preeclampsia was confirmed on day 15 based on urine protein levels and blood pressure measurement. After day 15, intraperitoneal administration of L-NAME was discontinued. Daily administration of 50 - 1000 mg/kg b.w of Dabino pulp extract began on day 16 until day 20 of euthanasia.

3.1. Ethical approach

All animals produced were conducted according to natural institutes of health guide for the care and use of laboratory animals and were approved by the Institutional Animal Care and Use Committee (IACUC) of Imo State University under the ethical number (IMSU/FBS/2023/021).

3.2. Collection of placental samples

After treatment for twenty-eight days, the four albino rats per group were fasted overnight (12 hours), weighed and anaesthetized by exposure to chloroform. The rats were sacrificed painlessly and placental samples collected. Placental samples (4 g) were taken in full thickness after placental delivery and washed with normal saline to prevent maternal contamination. Placental samples were then wrapped in aluminum foil and stored at -80 °C until analysis. For analyses, at least 1.5-g placental samples were tamponaded with 7 mL phosphate in a 10-mL tube and homogenized 10 000 cycle/min by Heidolph Diax 900 homogenizer (Rel Assay, Gaziantep, Turkey). After homogenization, placental tissue samples were taken into centrifuge tubes and analyzed (Ozturk et al., 2011).

3.3. Measurement of litter and maternal characteristics

Litter and maternal characteristics was measured using meter rule and weighing balance. Determination of diastolic blood pressure (DBP) and systolic blood pressure (SBP) of the Wistar rat was measured with a tail cuff sphygmomanometer (CODA, Kent Scientific, USA). All rats were kept warm by maintaining their temperature at 38°C to adapt to the experimental conditions before the actual measurements were performed (Wu & Xiao, 2019).

3.4. Measurement of Placental Antioxidants

Phosphate buffered saline was tested on placental tissue samples to buffer ratio of 1:4. Using the thiobarbituric acid reactive substances approach, the generation of lipid peroxidation products (malondialdehyde-MDA) was used to detect the damage to the lipid membrane (Draper & Hadley, 1990). The inhibition of adrenaline auto-oxidation was measured as absorbance at 480 nm to determine the activity of superoxide dismutase (SOD) (Bannister & Calabrese, 1987). The rate at which hydrogen peroxide decreased at 240 nm was used to quantify the activity of the enzyme catalase (CAT) (Aebi, 1984).

3.5. Measurement of Placental Cytokines

The levels of serum and placental cytokines (IL-6, TNF-a, IFN-g) was quantified by a commercial ELISA kit (Bio-Rad, USA) according to the manufacturer's instructions (Wu & Xiao, 2019). Homogenized placental samples were run in individual assay kits for each of the following analytes: IL-6 (high sensitivity kit), TNF-a (high sensitivity kit) and IFN-g (high sensitivity kit). Additional Phosphate buffers were used for any additional required dilutions. For IL-6, all lysates were analyzed at 3X and 12X dilutions. For TNF-a and IFN-g, all lysates were analyzed neat and at a 3X dilution (Sjaarda et al., 2018).

3.6. Measurement of Placental Growth Factors

The serum levels of VEGF, PLGF and sFlt-1 vascular endothelial growth factor receptor 1 were measured by Demeditec enzyme-linked immunosorbent assay kit: VEGF and PlGF ELISA assay kit was purchased from R&D Systems Inc. (DPG00) while sFlt1 (MBS2601616) reagent was purchased from My BioSource. The procedure was carried out following the instructions by the manufacturer (Batmomolin et al., 2020). To determine VEGF and PlGF in maternal serum, vacutainer tubes without additives were used. Samples were allowed to clot for 30 minutes at room temperature before centrifugation for 15 minutes at 1000 ×g. In order to determine serum sFlt-1, blood sample collected was stored

in refrigerator at 4°C for the night; then, the blood was centrifuged for 10 minutes at 1000 ×g and stored at −80°C until assayed. Samples were run in duplicate.

3.7. Data Analysis

Statistical Package for Biological and Social Sciences (SPSS) Inc. 27.0 Software program was used. Mean values (M) ± SD were calculated and one-way analysis of variance (ANOVA) was performed for multiple comparison. A $p \leq 0.05$ was considered statistically significant.

4. Result of pilot study

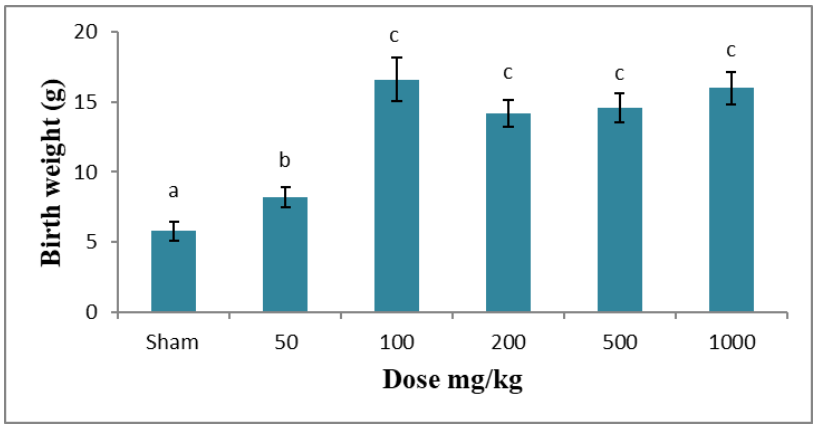

Figure 1A Pilot study showing the dose that gave effective treatment on birth weight for *Phoenix dactylifera* fruit extract

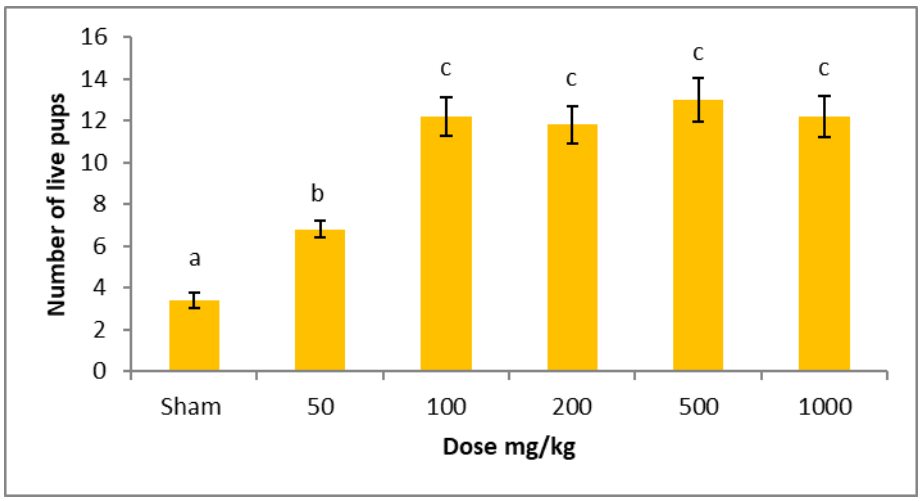

Figure 1B Pilot study showing the dose that gave effective treatment on number of live pups for *Phoenix dactylifera* fruit extract

Table 2 Litter Characteristics of Albino Rats

Treatment Groups	Total pups	Live pups	Still born	Birth weight (g)	Crown rump length (cm)
Group I	14.75±1.50[a]	14.00±1.41[a]	0.75±0.96[a]	3.68±0.17[a]	3.33±0.10[a]
Group II	13.75±1.71[a]	6.25±1.50[b]	7.50±1.91[b]	2.75±0.13[b]	2.88±0.05[b]
Group III	13.75±0.96[a]	12.75±1.89[a]	1.00±0.70[a]	3.45±0.17[a]	3.38±0.19[a]
Group IV	14.00±1.63[a]	13.25±1.25[a]	0.75±0.50[a]	3.78±0.13[a]	3.53±0.22[a]

Data are (M±S.D) of four determinations (n=4). Values bearing different superscript letters (a, b) are significantly different ($p<0.05$) down the column when compared to groups I and II.

Table 3 Maternal Characteristics of Albino Rats

Treatment groups	SBP	DBP	HBR	Weight gain	Feed intake	Placental weight (g)	Diuresis (ml/day)
Group I	122.75±2.63[a]	75.00±4.69[a]	289.25±16.82[a]	67.18±5.00[a]	621.75±12.39[a]	0.55±0.03[a]	4.00±0.14[a]
Group II	142.25±5.56[b]	91.75±3.59[b]	417.25±27.89[b]	39.65±3.47[b]	474.50±18.00[b]	0.43±0.03[b]	2.53±0.1[b]
Group III	127.50±3.79[a]	80.00±2.71[a]	294.00±8.04[a]	54.58±3.34[c]	563.50±18.30[c]	0.52±0.02[a]	4.78±0.1[c]
Group IV	125.50±6.46[a]	83.25±3.95[c]	297.75±16.68[a]	61.50±3.44[a]	663.25±16.82[c]	0.60±0.02[c]	3.93±0.1[a]

Data are (M±S.D) of four determinations (n=4). Values bearing different superscript letters (a, b) are significantly different ($p<0.05$) down the column when compared to groups I and II.; N/B- SBP- systolic blood pressure; DBR- diastolic blood pressure, HBR- heart beat rate.

Table 4 Effects of *Phoenix dactylifera* fruit Extract on Placental Antioxidant Parameters

Treatment groups	SOD (µ/mg tissue)	CAT (nmol/mg)	MDA (pg/mg tissue)
Group I	74.80±5.06[a]	179.15±8.34[a]	5.85±0.33[a]
Group II	46.22±4.60[b]	102.10±5.96[b]	11.75±0.95[b]
Group III	50.03±4.56[b]	130.48±7.89[c]	9.40±0.29[c]
Group IV	66.65±4.15[c]	175.33±13.23[a]	7.33±0.35[c]

Data are (M±S.D) of four determinations (n=4). Values bearing different superscript letters (a, b) are significantly different ($p<0.05$) down the column when compared to groups I and II.; N/B- SOD- superoxide dismutase; CAT- catalase, MDA- malondialdehyde; µ/mg tissue- micromole per milligram tissue; nmol/mg- nanomoles per milligram; pg/mg tissue- picogram per milligram tissue.

Table 5 Effects of *Phoenix dactylifera* fruit Extract on Placental Cytokine Parameters

Treatment groups	TNF-α (pg/mg tissue)	IL-6 (pg/mg tissue)	IFN (pg/mg tissue)
Group I	6.68±0.33[a]	2.15±0.37[a]	6.40±0.79[a]
Group II	17.38±0.98[b]	0.93±0.15[b]	15.90±0.98[b]
Group III	13.75±0.85[c]	0.93±0.21[b]	13.18±1.20[c]
Group IV	8.95±0.58[c]	1.75±0.13[c]	8.90±0.51[c]

Data are (M±S.D) of four determinations (n=4). Values bearing different superscript letters (a, b) are significantly different ($p<0.05$) down the column when compared to groups I and II.;N/B- TNF- tumor necrosis factor (TNF)-α; IL-6- interleukin-6 (IL)-6, IFN- interferon; pg/mg tissue- picogram per milligram tissue.

Table 6 Effects of *Phoenix dactylifera* fruit Extract on Placental Growth Factors

Treatment groups	VEGF (ng/ml)	PlGF (ng/ml)	sFLT-1 (ng/ml)
Group I	468.00±18.94[a]	68.33±2.72[a]	252.25±7.63[a]
Group II	216.00±9.23[b]	26.53±2.95[b]	760.75±13.62[b]
Group III	325.00±11.75[c]	43.50±2.20[c]	612.25±8.10[c]
Group IV	387.00±11.92[c]	63.40±3.48[c]	430.50±10.63[c]

Data are (M±S.D) of four determinations (n=4). Values bearing different superscript letters (a, b) are significantly different ($p<0.05$) down the column when compared to groups I and II. N/B- VEGF- vascular endothelial growth factor; PlGF- placental growth factor, sFLT-1- soluble vascular endothelial growth factor receptor 1; ng/ml - nanogram per milliliter.

5. Discussions

According to Pinheiro et al. (2013), preeclampsia is typified by elevated levels of proinflammatory cytokines in the mother, such as TNF-α, IFN, and IL-6 as well as distorted pregnancy outcomes. Excessive inflammation has been shown to be a major factor in the pathophysiology and etiology of preeclampsia in earlier studies (Hartley, Ferguson & Moffett, 2015). A growing body of research indicates that controlling inflammation could be a useful treatment for treating and preventing pregnancy-related issues.

Result of the pilot study showed that 100 mg/kg/day of *Phoenix dactylifera* fruit extract was the first dose that gave the best treatment on the birth weight in rats (Figure 1A). Also, result of the number of live pulps showed that 100 mg/kg/day of *Phoenix dactylifera* fruit extract was the first dose that gave the best treatment in rats (Figure 1B) which was the reason for administering this dosage to rats.

The litter characteristics of albino rats are showed in Table 2 revealed that all the parameters except total pups of rats in group II were negatively affected. Treatment with 20 mg/kg b.w of losartan drug and 100 mg/kg *Phoenix dactylifera* fruit extract significantly reversed this effect, although *Phoenix dactylifera* fruit extract had a better positive effect on the litter characteristics than losartan drug which shows the ability of *Phoenix dactylifera* fruit extract to enhance protection of both the pregnant rat and their fetus while inside the embryo. Findings are in line with the studies of da Silva et al. (2020) and Wu and Xiao (2019) who reported significant increase ($p<0.05$) in the number of live pups of preeclampsia-induced rats that were administered with Acai seed extract (ASE) and high dose of *Uncaria rhynchophylla* alkaloid extract respectively.

In this study, administration of 20 mg/kg b.w of losartan drug and 100 mg/kg b.w *Phoenix dactylifera* fruit extract significantly reduced systolic blood pressure, diastolic blood pressure and heart beat rate (Table 3). Similar result was reported by Salsabila et al. (2023), and Atoe and Idu (2022) who stated that methanolic leaf extract of three plants (*Jatropha cacus, Secamone afzelii* and *Alchonnea cordifolia*) administered at three different concentrations significantly decreased ($p<0.05$) the systolic and diastolic blood pressures at third trimester and post-partum in preeclampsia-induced rat. This could be the result of the presence of macrominerals, which are important for regulating blood pressure and include phenolic content, vitamin C, potassium, magnesium, e.t.c. (Vongpatanasin et al.., 2016).

The active component in *Phoenix dactylifera* fruit extract could be the reason behind the significant increase ($p<0.05$) in the weight gain, feed intake and placental weight of rats in group IV when compared with L-NAME group. Lee et al. (2003) state that drugs and diets reduce triglyceride levels in the body during lipid metabolism, which has an impact on weight gain in animals. Findings are in line with the report of da Silva et al. (2020) and Wu and Xiao (2019) who reported significant increase ($p<0.05$) in the placental weight of preeclampsia-induced rats that were administered with ASE and high, medium and low doses of *Uncaria rhynchophylla* alkaloid extract respectively.

The level of oxidative damaged and repair as a result of L-NAME and *Phoenix dactylifera* fruit extract was accessed by the concentrations of SOD, CAT and MDA antioxidants as shown in Table 4. Results showed that there was a significant increase ($p<0.05$) in the MDA of albino rats in group II when compared to group I indicating potential lipid oxidative damage; however, this was significantly reversed by 20 mg/kg b.w losartan drug and 100 mg/kg b.w *Phoenix dactylifera* fruit extract. On the contrary, there was a significant decrease ($p<0.05$) in the SOD and CAT of albino rats in group II when compared to group I while treatment with standard drug and dabino extract significantly increased their levels. *Phoenix dactylifera* fruit extract performed better on the CAT than losartan drug. Findings on the MDA levels agree with previous studies (da Costa et al., 2017; da Silva et al., 2018) that utilized other means to induce hypertension as well as

da Silva et al. (2020) who reported significant decrease in MDA levels of preeclampsia-induced rat treated with Acai seed extract. The antioxidant effect on the plasma may be attributed to a scavenger effect due to the presence of polyphenol; hence the activities of the antioxidant enzymes were not modified by administration of *Phoenix dactylifera* fruit extract (da Silva et al., 2020). That is to say, the antioxidant potentials of *Phoenix dactylifera* fruit extract might have aided in the protection of cardiac organ and enhanced endothelial function in preeclampsia-induced rats. More so, findings on the SOD and CAT obtained in this study are dissimilar to the report da Silva et al. (2020) who reported non-significant effect on the SOD and CAT of ASE treated preeclampsia-induced rats.

In this study, we found a significant increase ($p<0.05$) in the levels of TNF-α and IFN but a significant decrease ($p<0.05$) in the levels of IL-6 of albino rats induced with L-NAME preeclampsia when compared to control (Table 5). Furthermore, administration of both 100 mg/kg b.w *Phoenix dactylifera* fruit extract and 20 mg/kg b.w losartan drug significantly reversed the levels of TNF-α and IFN when compared with group II. Only the 100 mg/kg b.w *Phoenix dactylifera* fruit extract significantly increased the levels of IL-6 when compared with group II. The possible reason for the significant increase in the pro-inflammatory cytokines (TNF-α and IFN) in the L-NAME preeclampsia group could be due to upregulation process which requires numerous signal or as a result of the plasma content (Ambarwati et al., 2017). Findings on the TNF-α agree with previous studies by Ambarwati et al. (2017) who reported significant increase ($p<0.05$) in rats induced with preeclampsia and significant decrease ($p<0.05$) when *P. granatum* extract was administered at doses of 28 and 56 ppm respectively. Also, results of the levels of TNF-α and IFN agree with Wu and Xiao (2019) who reported significant decrease ($p<0.05$) in the placental levels of TNF-α and IFN of preeclampsia-induced rats treated with *Uncaria rhynchophylla* alkaloid extract at high and medium dosage.

These results suggest that a number of soluble compounds or factors that can cause pro-inflammatory cytokine upregulation are present in the plasma of pre-eclamptic patients. According to earlier research, fetal cells, pro-inflammatory cytokines (IL-6, IFN, and TNF-a), chemokines (IL-8, IP-10, and MCP-1), and adhesion molecules (ICAM-1 and VCAM-1) are all found in the plasma of preeclampsia (Ganshirt et al., 1998; Swinkels et al., 2002; Wataganara & Bianchi, 2004). This suggests that in L-NAME-induced pre-eclampsia, the active components of *Phoenix dactylifera* fruit extract, such as phenolic, flavonoid, saponins, and alkaloids, can inhibit the modulation signal of pro-inflammatory cytokines. These results are corroborated by other research (Afaq et al., 2005; Ahmed et al., 2005; An et al., 2015; Schubert, Neeman & Resnick, 2002) which described how pomegranate extract inhibited transcription factors (NF-kB) to help endothelial cells accumulate pro-inflammatory cytokines.

The result of the serum levels of placental growth factors (VEGF, PLGF and sFlt-1 in this study is shown in Table 6. There was a significant decrease ($p<0.05$) in the levels of VEGF and PlGF and a significant increase ($p<0.05$) in the sFLT-1 levels of albino rats in group II when compared to group I. Treatment with 20 mg/kg b.w losartan drug and 100 mg/kg b.w *Phoenix dactylifera* fruit extract effectively reversed the damage with *Phoenix dactylifera* fruit extract performing better than the standard drug although both of them did not reverse the serum levels of placental growth factors to normalcy. The significant increase in VEGF and decrease in sFlt-1 in rats administered *Phoenix dactylifera* fruit extract implies that it support growth and development of the fetus (Tang et al., 2019). Findings on the VEGF are not in line with the study by Tang et al. (2005) who reported significant increase in VEGF under preeclampsia condition. The significant decrease ($p<0.05$) in the VEGF of group II implies that it might not support the induction of thrombomodulin expression in cells and by implication does not help in the upregulatory action of the renal thrombomodulin. According to Calnek and Grinnell (1998), the failure of VEGF to induce thrombomodulin negatively affects the upregulation of renal under preeclampsia condition. Results obtained on sFlt-1 are similar to Batmomolin et al. (2020) as well as the study by Ambarwati et al. (2017) who reported that the administration of *P. granatum* extract at a dose of 56 ppm was capable of reducing the levels of sFlt-1 significantly compared with the group of human umbilical vein endothelial cells (HUVECs) exposed to preeclampsia plasma ($P < 0.05$), and reached the levels comparable to controls ($p > 0.05$). These results validate the anti-angiogenic properties of the active components in *Phoenix dactylifera* fruit extract, most likely due to the phenolic and flavonoid compounds' inhibition of NF-kB signal (Eddy et al., 2018). This outcome could go any of two ways. First, sFlt-1 levels in the maternal circulation are lowered when *Phoenix dactylifera* fruit extract was given, but sFlt-1 was still created and delivered by the placenta into the maternal circulation (Batmomolin et al., 2020). According to Palmer et al. (2017), placental release into the mother's bloodstream is the main source of sFlt-1. Second, *Phoenix dactylifera* fruit extract did not prevent elevated blood pressure and excessive inflammation in a rat model of preeclampsia through the angiogenesis pathway; this needs more investigation (Batmomolin et al., 2020). The results showed that *Phoenix dactylifera* fruit extract corrected the angiogenic imbalance that was caused in preeclampsia-induced rats by rescuing aberrant uteroplacental angiogenic state in the preeclampsia rat model.

6. Conclusion

This study demonstrated positive modulatory effects of *Phoenix dactylifera* fruit extract on pregnancy parameters and placental specific parameters of preeclampsia induced rats. Therefore, *Phoenix dactylifera* fruit extract can be utilized as a potential agent for the management of hypertension and placental related complications during pregnancy.

Compliance with ethical standards

Disclosure of conflict of interest

Authors have declared that no competing interests exist.

Statement of ethical approval

All animals produced were conducted according to natural institutes of health guide for the care and use of laboratory animals and were approved by the Institutional Animal Care and Use Committee (IACUC) of Imo State University under the ethical number (IMSU/FBS/2023/021).

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