LASU Journal of Medical Sciences
Official Publication of the Faculty of Basic Medical Sciences and Faculty of Basic Clinical Sciences
Lagos State University College of Medicine, Ikeja
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Research Article

Gestational Exposure to Citrus Limon Linn Juice, Oleic, Palmitic and Stearic Acids Reduce Placental Efficiency and Foetal Morphometric Indices in Wistar Rats

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Keywords:
Citrus limon juice, Hypolipidemia, Placental efficiency, Foetal morphometry

ABSTRACT
Objective: Foetal outcome is associated with maternal and intrauterine environment. The relationship between some hypolipidemic agents on placental and foetal outcomes was investigated.
Methods: *Citrus limon* Juice (CLJ) (1.00 ml/kg), oleic acid (0.31 ml/kg), palmitic acid (0.26 ml/kg) stearic acid (0.19 ml/kg) and distilled water (1.00 ml/kg) were administered orally on gestation days 1-20. Non-survival Caesarean section was done to obtain placentas and foetuses. Foetal weight, crown-rump length, abdominal circumference, placental thickness, lipid profile, Small Neutral Amino Acids (SNAAT-1), Glucose Transporter (GLUT-1), Fatty Acid Transport Protein (FATP-1) and histology were assessed. Data were analysed with ANOVA at $p<0.05$.
Results: The CLJ and stearic acid significantly reduced weight of female foetuses compared with control. Crown-rump length significantly reduced in CLJ, oleic acid and stearic acid groups as compared with control. Male abdominal circumference significantly reduced in all groups compared with control. Placental thickness significantly increased in CLJ group compared with control. Placental lipid profile significantly reduced in all groups compared with control. Placental SNAAT-1 significantly reduced in CLJ, oleic acid and stearic acid groups relative to control. Placental GLUT-1 significantly reduced in CLJ and oleic acid groups relative to control. Male placental FATP-1 significantly reduced in CLJ group compared with control. Placental sections from CLJ group showed inflammatory cells infiltration and decreased fat deposition.
Conclusion: Maternal Citrus limon juice administration reduced placental nutrient transporters and efficiency, thereby reducing foetal morphometric indices. This effect which was more pronounced in male foetuses is linked to the oleic and stearic acids.

INTRODUCTION

Maternal internal environment from which a developing foetus derives its nutrients is affected by exposure to substances ingested by the mother, oxidative status as well as hormonal imbalances which are able to modify the foetal growth and development.[1] Maternal nutritional imbalance and metabolic disruption during critical periods of development have been reported to cause long term health effects in offspring,[2] and increase the risk of diseases in later life.[3] Foetal morphometric indices such as birth weight, crown-rump length, abdominal and head circumferences are used as markers that represent intrauterine growth and placental efficiency.[4] Previous reports have shown that reduced birth weight may be an indication of failure of placenta to adapt to changes in maternal internal environment,[5] which has been a major cause of cardiovascular diseases,[6] obesity [7] and diabetes [8] in adult life. Hence, foetal outcome is dependent on maternal environment and placental efficiency.

The placenta serves as the primary interface between the foetus and the mother.[9] It maintains foetal growth by facilitating transfer of nutrients substrate such as glucose, amino acids and fatty acids as well as wastes between the maternal and foetal circulations.[9] The transport processes of these nutrients depend on placental morphology and vascularity [10] as well as physiological and biochemical characteristics such as receptors, enzymes, hormones and nutrients transporters.[11] Studies have shown that maternal insults are transferred to the foetus via alterations in placental functions,[12] thereby affecting the growth and development of foetal tissues.[13] Apart from the maternal insults, growth of the foetus is also regulated by the availability of nutrients in maternal circulation, which are transferred into foetal circulation through the placenta.[10] The placenta must

therefore function optimally in order to supply nutrients to the foetus efficiently.

Herbal products are widely used in the developing countries for health care needs, not only because they are inexpensive but due to better cultural acceptability and compatibility with the human body system.[14] According to the World Health Organisation,[15] 80% of the populace living in Africa use herbal medicines for primary health care. Also, in United States of America, herbal products are used by approximately 20% of the population.[16]

Citrus limon fruit (lemon), an example of herbal products is from citrus family Rutacea, it is widely cultivated globally.[17] In Nigeria, it is locally known as "lemonu/osan gaingain" (Yoruba), "lemuntsami" (Hausa) and "oroma-nkrisi" (Igbo).[18] The juice and its major bioactive components oleic, palmitic and stearic acids have been reported to lower low-density lipoprotein and total cholesterol due to its hypolipidaemic property.[19] It also promotes platelet aggregation thereby enhancing wound healing.[20] Its ability to cause weight loss [21] and prevent kidney stone [22] has also been documented. It is commonly consumed during pregnancy to alleviate morning sickness, which can last for several months or throughout the pregnancy in some women [23] and to prevent severe bleeding especially during the first trimester.[24] Nevertheless, it is a known hypolipidemic agent with potent effect on body fat loss.[21] The ability of hypolipidemic agents to alter internal milieu as well as transport nutrients raises a concern about the growth and development of the foetus which is dependent on optimal transport of nutrients across the placenta. There is dearth of scientific knowledge on the association between Citrus limon juice, its bioactive constituents, placental and foetal outcomes. The study was therefore designed to investigate the effects of maternal administration of *Citrus limon* juice, oleic, palmitic and stearic acids on placental efficiency and foetal morphometric indices in Wistar rats.

METHODS

Ethical approval was obtained from University of Ibadan, Animal Care and Use Research Ethics Committee, with voucher number UI-ACUREC/19/0014 and the experimental protocols and procedures in the study were conducted according to the guidelines of animal protection and welfare of the University of Ibadan, Nigeria and according to International Guide for the Care and Use of Laboratory Animals.[25]

Experimental animals

Ten male (180-200 g) and twenty female (100-150 g) Wistar rats obtained from the Central Animal House, College of Medicine, University of Ibadan were used for the study. The male rats were proven breeders, while the females were virgin rats with regular oestrous cycles. The rats were housed in the Postgraduate Animal House, Department of Physiology, College of Medicine, University of Ibadan, in aerated plastic cages and had access to feed and water *ad libitum*. Animals were acclimatized to the environmental condition of the animal house for two weeks before the start of the study. The rats were grouped as follows: Group 1 (Control) received 1.00 ml/kg of distilled water; Group 2 rats received 1.00 ml/kg of Citrus limon juice; Group 3 rats received 0.31 ml/kg of oleic acid; Group 4 rats received 0.26 ml/kg of palmitic acid; Group 5 rats received 0.19 ml/kg of stearic acid. All administrations were given via oral gavage from gestation day 1 to 20. Twenty-four hours maternal feed intake in the rats was measured by subtracting the leftover weight from the initial weight of the feed presented to the animals.[26]

Preparation of *Citrus limon* juice

Fresh *Citrus limon* fruits were obtained from a farm in Ibadan, the fruits were authenticated at Forestry Research Institute of Nigeria (FRIN) with voucher number FHI.110938. The fruits were washed, cut into halves and the juice contents were expressed into a glass beaker. The expressed juice was filtered using a clean sieve and the filtrate was collected into clean bottles. An aliquot of the filtrate was concentrated using a rotary evaporator in order to determine the concentration. The filtrate was administered to the animals at a dose of 1 mL/kg orally [27] which is 91 mg/kg body weight.

Reagents

Analytical grade 9-octadecenoic acid (oleic acid), octadecanoic acid (stearic acid) and n-Hexadecanoic acid (palmitic acid) used in the study were obtained from Sigma Aldrich chemicals.

Identification of individual constituents in CLJ

Analysis of individual constituents in CLJ was done using Gas Chromatography and Mass Spectrometry (GC-MS), with an Agilent 7890AGC interfaced to an Agilent 5973N mass selective detector. The peak numbers, relative abundance of constituents and retention time was recorded and data was retrieved via GC-MS solution software and National Institute Standards and Technology (NIST) library was used to point out the corresponding peaks.[28]

Mating procedure and determination of pregnancy

All female rats were mated overnight during proestrus phase with proven male breeders in ratio 2:1 (female to male). The vaginal lavage was examined under 40× objective lens of the light microscope (XS2 107, China) every morning following mating. Mating was confirmed by the presence of spermatozoa in the vaginal lavage. The day on which spermatozoa were observed in the vaginal lavage was designated as gestation day 1.

Caesarean section

At gestation day 20, non-survival Caesarean section was performed under thiopentone anaesthesia (50 mg/kg, i.p.).[29] The animals were surgically opened around the pelvic region to fully expose the gravid uterus, which was carefully removed from the root. The pups with its respective placentas were carefully dissected from the uterus. Foetal and placental morphometry were estimated. Anogenital distance was used to determine the sex of the foetuses with the aid of Vernier calliper and litters size of 6-8 was used for standardisation. Two placentas per sex and dam were fixed in 10% neutral buffer formalin for histological assessment, while other placentas were homogenised in phosphate buffer (pH 7.4) for lipid profile, placental nutrient transporters (GLUT-1, SNAAT-1 and FATP-1) analysis using ELISA

technique.

Determination of placental morphometric indices

The placental weight was determined by weighing the placenta of each foetus on a digital electronic weighing scale (Lisay, China). Placental volume was measured using Archimedes principle by dropping each placenta into a measuring cylinder containing a known volume of phosphate buffer solution. The displaced volume of phosphate buffer was recorded as the volume of placenta. Placental thickness, circumference and chorionic surface area were measured and calculated as previously described.[30]

Determination of foetal morphometric indices

Foetal morphometry was carried out immediately after the foetus was obtained by Caesarean section. The foetuses were weighed on an electronic scale (Lisay, China). The head diameter was measured from one ear to the other ear, abdominal diameter was measured at the centre of the waist, Ano-Genital Distance (AGD) was measured from the genital ridge to the anus, crown-rump length was measured from the tip of the nose to the root of the tail.

All measurements were done using a digital Vernier calliper (Mitutotyo, Japan), while the pups were gently but firmly held. Head circumference ($2\pi r$), abdominal circumference ($2\pi r$) and anogenital distance index (AGD/body weight1/3) [31] were calculated from the data obtained.

ELISA Principle used in the study

Placental lactogen, SNAAT-1, GLUT-1 and FATP-1 were assayed using direct competitive and sandwich principle as previously described.[32] Briefly, an aliquot of placental homogenate is incubated in wells coated with monoclonal antibody conjugated with horseradish peroxidase. The amount of bound peroxidase is proportional to the concentration of analytes in the homogenate.

Preparation of histological slides

The samples were fixed in 10% neutral buffer formalin for at least 5h, immediately after it was collected from the animal. After which, each tissue was placed in 70, 80, 90 and 95% and two changes of absolute alcohol for 1h each in order to dehydrate them. They were then placed in two changes of xylene for 1h each. On removing it from xylene, it was placed in wax bath, at least two changes for h each and it was embedded in paraffin wax. The tissue was then trimmed nicked and sectioned at 3–5 micron with a microtome. The section was floated with 20% alcohol on water at a temperature of 5°C below paraffin wax melting point. The section was picked with a clean, grease-free microscope slide and water was drained and placed on microscope slide for at least 1h. Staining was done with hematoxylin and eosin for the purpose of determining the general morphology. A photomicrograph of the sections was made in order to observe morphological changes.

Statistical analysis

Data were expressed as mean ± Standard Error of Mean (SEM) and the differences in means were compared by analysis of variance (ANOVA). $P < 0.05$ was considered statistically significant. GraphPad prism 7.01 (GraphPad software, Inc, U.S.A.) was used to analyse the data.

RESULTS

The GC-MS analysis of Citrus limon juice revealed presence of 12 compounds, of which the major constituents are oleic acid, palmitic acid and stearic acid in terms of percentage of 30.48%, 25.81% and 19.44% respectively (Table 1).

Maternal feed intake was significantly reduced in CLJ and SA groups during the first and second week of administration when compared with control (Table 2).

Crown-rump length and abdominal circumference of male foetus was significantly reduced in all the groups when compared with control (Table 3), while crown-rump length of female foetus was significantly reduced in all the groups and foetal weight was significantly reduced in CLJ and SA groups when compared with control (Table 3). Placental thickness of male foetus was significantly increased in CLJ group when compared with control (Table 4a), while that of female foetus was significantly increased in CLJ and OA groups when compared with control (Table 4b). Male and female foetal placental lipid profile was significantly reduced in all the groups when compared with control (Table 5). Male placental lactogen was significantly reduced in all the groups when compared with control (Figure 1), while it was significantly reduced in CLJ group in female foetus as compared with control (Figure 1). Male and female placental SNAAT-1 was significantly reduced in CLJ, OA and SA groups when compared with control (Figure 2). Male placental GLUT-1 was significantly reduced in CLJ, OA and SA groups when compared with control (Figure 3) and it was however significantly increased in PA group when compared with control (Figure 3). Placental FATP-1 of male offspring was significantly reduced in CLJ when compared with control but increased in OA, PA and SA groups when compared with control (Figure 4). Histology of placental tissue shows reduced fat deposits and infiltration of inflammatory cells in CLJ group (Figure 5 and Figure 6).

Table 1: Nomenclature of individual constituents in Citrus limon juice

Pk#	RT	Area%	Library/ID	Ref#	CAS#	Qual
1.	5.975	3.44	Hydrazine, 1,1-dimethyl-	280	000057-14-7	4
			Hydrazine, 1,1-dimethyl-	279	000057-14-7	4
			Urea	298	000057-13-6	4
2.	38.928	25.81	n-Hexadecanoic acid	107549	000057-10-3	99
			Tetradecanoic acid	84455	000544-63-8	90
			Tridecanoic acid	72646	000638-53-9	86
3.	40.027	30.48	Oleic acid	129338	000112-80-1	99
			9-Octadecenoic acid, (E)-	129353	000112-79-8	99
			Cis-Vaccenic acid	129339	000506-17-2	99
4.	40.177	19.44	Octadecanoic acid	131262	000057-11-4	95
			Pentadecanoic acid	95855	001002-84-2	93
			Octadecanoic acid	131261	000057-11-4	80
5.	40.239	5.54	Azetidine, 1-benzoyl-3-ethenyl-	51005	118973-03-8	27
			Pyrazol-3-amine, 1-(4-methylphenyl)	50914	1000272-83-6	16
			2-Azetidinone,3-(1-methylethylidene)-1-phenyl-	51025	068695-52-3	14
6.	40.287	3.32	N-(2-Chloroethyl) benzamide	48211	026385-07-9	43
			Quinoline, N-benzoyl-1,2,3,4-tetra hydro-	91604	028748-92-7	10
			Thiazolo[3,2-a] [1,3,5]-triazin-6(7H)-one,	124842	088696-71-3	9
			3,4 (2H)-dihydro-7-hydroxymethyl-7-methyl-3-phenyl-			

Pk=Peak; RT= Retention Time; Ref#= Reference Number, Qual= Quality

Table 2. Effects of Citrus limon juice (CLJ) and its major components on maternal feed consumption in pregnant Wistar rat dams

GROUP	CONTROL (g)	CLJ (g)	OA (g)	PA (g)	SA (g)
WEEK	181.57±1.5	63.14±1.10*	73.43±3.83	87.00±1.39	58.00±3.11^{*,c}
WEEK	282.29±2.11	67.71±1.07*	73.71±1.82	83.71±1.95	58.43±3.64^{*,a,c}
WEEK	381.83±1.45	80.33±1.06	84.83±1.7	389.67±1.22	71.00±1.73^{c}

Data presented as mean± SEM, n=5, *p<0.05 in comparison to control, a=p<0.05 in comparison to CLJ, c=p<0.05 in comparison to PA. CLJ=Citrus limon juice; OA=Oleic Acid; PA=Palmitic Acid; SA=Stearic Acid.

Table 3: Male and female foetal morphometric indices of pregnant Wistar rats following treatment

	MALE					FEMALE				
GROUPS	FW (g)	HC (mm)	AC (mm)	CRL (mm)	AGDi (mm/g3)	FW (g)	HC (mm)	AC (mm)	CRL (mm)	AGDi (mm/g3)
CONTROL	2.50±0.08	10.56±0.20	13.58±0.32	74.71±4.90	1.84±0.10	2.42±0.09	10.45±0.19	13.26±0.23	74.50±3.06	1.00±0.09
CLJ	2.14±0.01	10.76±0.47	12.53±0.33*	59.60±1.10*	1.81±0.11	2.11±0.04*	10.87±0.17	12.65±0.36	59.17±1.69*	1.02±0.11
OA	2.24±0.09	11.16±0.26	12.50±0.29*	57.00±2.42*	1.88±0.12	2.32±0.09	10.99±0.18	12.13±0.34	54.90±1.77*	1.04±0.08
PA	2.34±0.04	11.11±0.54	12.20±0.19*	57.75±0.61*	1.72±0.07	2.27±0.061	11.35±0.70	12.39±0.30	59.10±1.65*	1.04±0.08
SA	2.07±0.04	11.59±0.69	12.47±0.39*	57.00±2.55*	1.77±0.08	2.07±0.03*	11.30±0.52	12.34±0.35	54.70±1.96*	1.06±0.08

Data are presented as mean± SEM, n=5, *p<0.05 compared with control. CLJ = Citrus limon juice; OA = oleic acid; PA = palmitic acid; SA = stearic acid, FW = Foetal Weight; HC = Head Circumference; AC = Abdominal Circumference; CRL = Crown-Rump Length; AGDi =Anogenital Distance index

Table 4a: Male foetal placental morphometric indices of pregnant Wistar rats

GROUPS	CONTROL	CLJ	OA	PA	SA
LS	7±0.4	7±0.5	8±0.3	8±0.2	7±0.4
FW(g)	2.3±0.1	2.1±0.0	2.2±0.1	2.3±0.0	2.1±0.0
PW(g)	0.4±0.	00.5±0.0	0.4±0.0	0.4±0.0	0.4±0.0
PT(mm)	2.3±0.2	3.3±0.1*	2.6±0.1	2.6±0.1	2.3±0.1
PV(mm3	0.5±0.0	0.5±0.0	0.5±0.0	0.5±0.0	0.5±0.0
PCSA(cm2)	2.1±0.1	2.2±0.1	2.2±0.1	2.1±0.0	2.2±0.1
PC	0.2±0.0	0.2±0.0	0.2±0.0	0.2±0.0	0.2±0.0
FPR	5.8±0.2	5.1±0.3	5.3±0.2	5.8±0.3	5.3±0.2

Data are presented as mean ± SEM. n = 5. *p<0.05 when compared with the control group. CLJ = Citrus limon Juice. OA = Oleic Acid. PA = Palmitic Acid. SA = Stearic Acid. LS = Litter Size. FW = Foetal Weight. PW = Placental Weight. PT = Placental Thickness, PV = Placental Volume. PCSA = Placental Chorionic Surface Area. PC = Placental Coefficient. FPR = Foeto-Placental Ratio.

Table 4b: Female foetal placental morphometric indices of pregnant Wistar rats

GROUPS	CONTROL	CLJ	OA	PA	SA
LS	7±0.4	7±0.5	8±0.3	8±0.	27±0.4
FW(g)	2.4±0.1	2.1±0.0*	2.3±0.1	2.3±0.1	2.1±0.0*
PW(g)	0.4±0.0	0.5±0.0	0.5±0.0	0.4±0.0	0.4±0.0
PT(mm)	2.2±0.2	3.6±0.1*	2.9±0.2*	2.5±0.2	2.1±0.1
PV(mm3)	0.5±0.0	0.4±0.00.	5±0.0	0.5±0.0	0.5±0.0
PCSA(cm2)	2.1±0.0	2.0±0.12.	2±0.1	2.2±0.1	2.1±0.1
PC	0.2±0.0	0.2±0.00.	2±0.0	0.2±0.0	0.2±0.0
FPR	6.2±0.4	4.8±0.2*	5.2±0.3	5.5±0.3	6.0±0.2

Data are presented as mean ± SEM. n = 5. *p<0.05 when compared with control. CLJ = Citrus limon Juice. OA = Oleic Acid. PA = Palmitic Acid. SA = Stearic Acid. LS = Litter Size. FW = Foetal Weight. PW = Placental Weight. PT = Placental Thickness, PV = Placental Volume. PCSA = Placental Chorionic Surface Area. PC = Placental Coefficient. FPR = Foeto-Placental Ratio.

Table 5: Male and Female foetal placental lipid concentration of pregnant Wistar rats

	MALE				FEMALE			
GROUPS	**TC (mg/dL)**	**LDL-C (mg/dL)**	**HDL-C (mg/dL)**	**TG (mg/dL)**	**TC (mg/dL)**	**LDL-C (mg/dL)**	**HDL-C (mg/dL)**	**TG (mg/dL)**
CONTROL	253.92±0.10	239.29±0.10	2.20±0.22	62.15±2.66	252.52±0.02	236.42±0.02	2.46±0.59	68.21±1.49
CLJ	162.90±0.09*	151.86±0.09*	0.98±0.29	50.26±3.36*	223.92±0.01*	216.92±0.06*	0.67±0.11*	81.67±0.10*
OA	196.04±0.08^{*,a}	186.67±0.08^{*,a}	0.24±0.05	46.45±0.93*	163.37±0.01^{*,a}	145.30±0.01^{*,a}	1.37±0.29^{*,a}	84.59±0.16^{*,a}
PA	137.23±0.12^{*,a,b}	117.44±0.12^{*,a,b}	0.38±0.17	84.05±3.99^{*,a,b}	142.83±0.06^{*,a,b}	128.46±0.06^{*,a,b}	0.31±0.08^{*,b}	71.58±0.25^{*,a,b}
SA	187.17±0.10^{*,a,b,c}	166.57±0.10^{*,a,b,c}	10.51±0.37^{*,a,b,c}	50.49±1.69^{*,c}	221.71±0.06^{*,a,b,c}	211.09±0.06^{*,a,b,c}	1.24±0.19^{*,a,c}	46.89±0.08^{*,a,c}

Data are presented as mean± SEM, n=5, *p<0.05 compared with control, a=p<0.05 compared with CLJ, b=p<0.05 compared with OA, c=p<0.05 compared with PA. CLJ= *Citrus limon* juice; OA=oleic acid; PA=palmitic acid; SA=stearic acid.

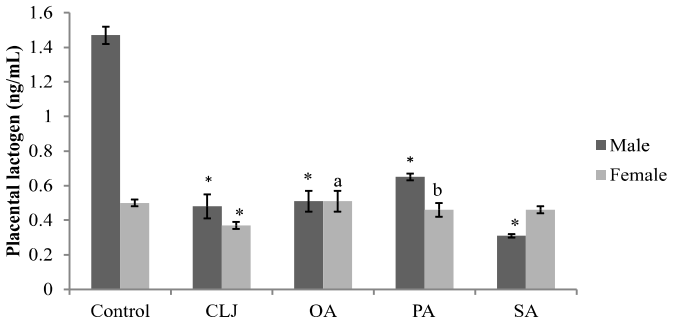

Figure 1: Foetal placental lactogen of pregnant Wistar rats
Data are presented as mean± SEM, n=5, *p<0.05 compared with control, a=p<0.05 compared with CLJ, b=p<0.05 compared with OA. CLJ=Citrus limon juice; OA=oleic acid; PA=palmitic acid; SA=stearic acid.

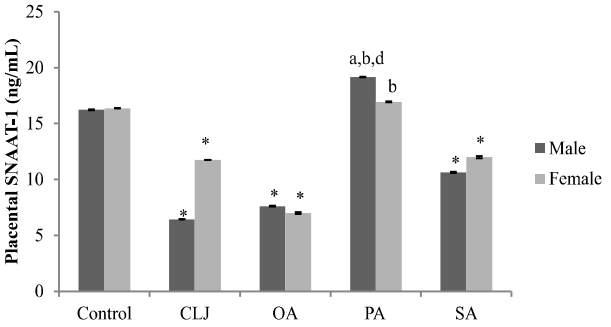

Figure 2: Foetal placental SNAAT-1 concentration of pregnant Wistar rats

Data are presented as mean± SEM, n=5, *p<0.05 compared with control, a=p<0.05 compared with CLJ, b=p<0.05 compared with OA, c=p<0.05 compared with PA. CLJ=*Citrus limon* juice; OA=oleic acid; PA=palmitic acid; SA=stearic acid.

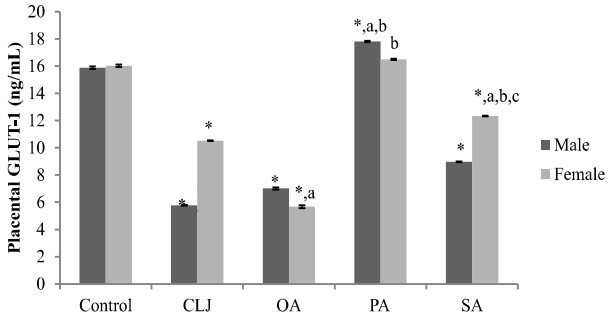

Figure 3: Foetal placental GLUT-1 concentration of pregnant Wistar rats

Data are presented as mean± SEM, n=5, *p<0.05 compared with control, a=p<0.05 compared with CLJ, b=p<0.05 compared with OA, c=p<0.05 compared with PA. CLJ=*Citrus limon* juice; OA=oleic acid; PA=palmitic acid; SA=stearic acid.

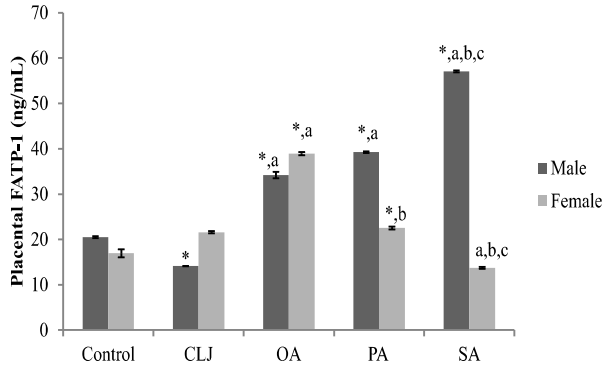

Figure 4: Foetal placental FATP-1 concentration of pregnant Wistar rats

Data are presented as mean± SEM, n=5, *p<0.05 compared with control, a=p<0.05 compared with CLJ, b=p<0.05 compared with OA, c=p<0.05 compared with PA. CLJ=*Citrus limon* juice; OA=oleic acid; PA=palmitic acid; SA=stearic acid.

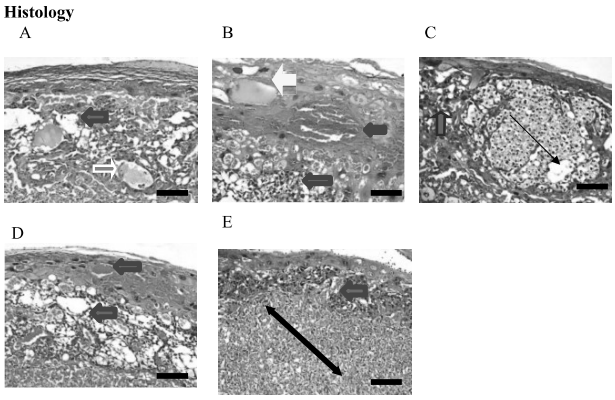

Figure 5: Photomicrographs of placenta sections from male foetus of control, Citrus limon juice and its major components treated pregnant Wistar rat dams

Tissues were stained with H&E and presented at ×100 magnification, Bar = 1.2mm. A) Control: Placental tissue show normal labyrinth zone, decidual cells appear normal with moderate fat deposits (blue arrow) and degenerating glycogen cells (green arrow). B) CLJ: Placental tissue show normal labyrinth zone, oedematous basal zone (yellow arrow) with moderate vascular congestion (red arrow). C) OA: Placental tissue show moderate presence of glycogen cells (slender arrow) and moderate fat deposits in the basal intervilous spaces (blue arrow).D) PA: Placental tissue show moderate vascular congestions in the labyrinth zone (red arrow) and abundant fat deposit (blue arrow). E) SA: Placental tissue show normal labyrinth zone (spanned arrow) and oedema in the basal layer (red arrow).CLJ (Citrus limon Juice), OA (Oleic Acid), PA (Palmitic Acid), SA (Stearic Acid).

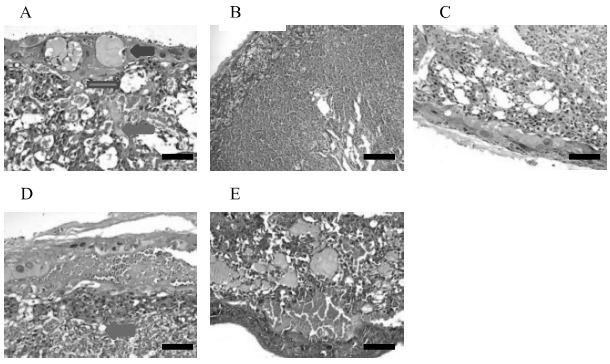

Figure 6: Photomicrographs of placenta sections from female foetus of control, Citrus limon juice and its major components treated pregnant Wistar rat dams

Tissues were stained with H&E and presented at ×100 magnification, Bar = 1.2mm. A) Control: Placental tissue show basal layer with moderate vascular congestions (red arrow), oedema (blue arrow) and fats deposits (green arrow). The decidual cells appear hyperplastic (blue arrow). B) CLJ: Placental tissue show labyrinth zone with severe vascular congestion (spanned arrow). C) OA: Placental tissue show basal layer with moderate fat deposit (green arrow), labyrinth zone appears oedematous with infiltration by inflammatory cells (black arrow). D) PA: Placental tissue shows infiltration of inflammatory cells (black arrow) and moderate vascular congestions in the basal layer. The labyrinth zone appears oedematous (blue arrow) with mild fat deposit and the decidual cells appear hyperplastic. E) SA: Placental tissue show basal layer with moderate oedema and moderate vascular congestions (red arrow), labyrinth zone appears moderately oedematous (blue arrow) with mild fat deposit (green arrow). The decidual cells appear hyperplastic.CLJ (Citrus limon Juice), OA (Oleic Acid), PA (Palmitic Acid), SA (Stearic Acid).

DISCUSSION

The foetal growth is usually evaluated by body weight and some body parts measurements mostly crown-rump length, head circumference and abdominal circumference.[33] Abdominal circumference for instance is very useful in assessing nutritional status in normal and altered states of foetal growth because the abdomen encompasses the liver and subcutaneous tissue in the area, which show reduction in size secondary to decrease in nutrient substrate and is shown to be associated with intrauterine growth restriction (IUGR).[33] In the present study there was reduction in nutrient transporters that are necessary for nutrient delivery to the foetus via the placenta, which in turn leads to reduction in female foetal weight as well as abdominal circumference and crown-rump length. Although it has been proven that male sex are more vulnerable to adverse pregnancy outcomes.[34] However, the observed reduction in the birth weight of female offspring in this study recapitulates other observations that show how female foetuses curtail their rate of growth as a survival strategy in preparation for multiple insults in future.[35,36]

Placenta is a critical organ for foetal growth and development.[12,13] Alterations in its morphology such as size, thickness and nutrient transport capability contribute to placental dysfunction.[10] Placental thickness indicates the adequacy of the villous tree arrangement in the placenta and the available blood volume of the foetus.[37] Increase in placental thickness is a useful predictor of adverse pregnancy outcomes such as low birth weight, rate of emergency Caesarean section deliveries, foetal growth restriction, abruptio placentae, pregnancy induced hypertension, and congenital anomalies.[38] In this study, histology of the placenta tissue shows infiltration of inflammatory cells and oedema which may be due to compensatory proliferation of the placenta as a result of the increase in its thickness, the results observed is in accordance with previous studies.[39,40]

Nutrient substrates such as glucose, amino acids, fatty acids and cholesterol are essential for optimal foetal growth.[41] Lipids are essential in pregnancy homeostasis,[42] placental lipid transfer to the foetus depends on lipid in maternal circulation and lipid alterations are capable of generating permanent changes in the structures and functions of the developing organs, which may reflect on their metabolism and post-uterine life.[43] Citrus limon juice, oleic acid, palmitic acid and stearic acid: all being hypolipidemic agents resulted in reduction of placental lipid profile, leading to reduced lipid delivery to the foetus accompany with the reduction in maternal feed intake and placental transporters thereby causing reduction in birth weight, which is a major predisposing factor to developing diseases in adulthood. Foetal glucose is derived exclusively from the mother, yet placental lactogen as well as transplacental glucose transport determines glucose delivery to the foetus.[44] Administration of Citrus limon juice and its most abundant constituents resulted into reduced concentration of male placental lactogen and GLUT-1, thereby reducing glucose transfer to the foetus. Placental transport of fatty acids is critical for foetal growth most especially in late gestation because fat deposition increases exponentially during this period.[45] In a typical pregnancy condition, maternal triglycerides increases gradually throughout gestation and are substrates for placental lipase which is associated with free fatty acids release for transport to the foetus,[42] this transport is regulated by fatty acid transporters such as fatty acid transport protein (FATP-1).[43]

In this study the concentration of male placental FATP-1 was reduced thereby resulting into altered birth morphometric indices, which is evident with the reduction in abdominal circumference and crown-rump length observed in the male foetus. Amino acids are important precursors for biosynthesis of proteins.[38] The concentration of free amino acids in the placental tissue is higher than foetal and maternal circulation, hence the foetal supply of amino acids is critically dependent on the transport capacity of the placenta via amino acids transporters.[46] In this study placental amino acid transporter (SNAAT-1) was reduced, thereby impacting on the amount of amino acids transfer to the foetus, which has been associated with low birth weight and intrauterine growth restriction.[47]

The ability of the placenta to maintain sufficient nutrient supply is commonly described as placental efficiency.[48] A previous study suggests that decreased oxygen and nutrients supply arise in placental insufficiency, as a result the foetus tends to redistribute blood to the brain at the costs of the liver glycogen and fatty acid storage thereby resulting in normal foetal brain growth but decline in the growth of abdominal circumference.[49] In this study, the observed reduction in foetal weight, crown-rump length and foeto-placental ratio in the female foetus as well as increase in placental thickness and lipid profile is associated with reduced transfer of nutrient substrates from placental to the foetal circulation, as a result of decline in the placental efficiency, which has been linked to upsurge in the incidence of intrauterine growth restriction.[5]

CONCLUSION

Maternal administration of Citrus limon juice and its major constituents (oleic, palmitic and stearic acids) resulted in reduced placental lipid profile and nutrient transporters thereby affecting placental efficiency and foetal morphometry. Placental and foetal outcomes observed in the study may be linked to the oleic acid and stearic acid constituents of Citrus limon juice.

ACKNOWLEDGEMENTS

This research received no specific grant from any funding agency, commercial or not-for-profit sectors.

Conflicts of Interest

None

Ethical Standards

All procedures contributing to this work comply with the ethical standards of the NIH guides on the care and use of laboratory animals (NIH Publication revised; No. 85–23 revised 1996) and have been approved by the Ethical committee, University of Ibadan.

REFERENCES

1. Fowden AL, Forhead AJ, Coan PM, Burton GJ. The placenta and intrauterine programming. J Neuroendocrinol. 2008;20:439–450.
2. Sutton EF, Gilmore LA, Dunger DB, Heijmans BT,

Hivert M, Ling C et al. Developmental Programming: State-of-the-science and future directions. Summary from a Pennington biomedical symposium. J Obesity (Silver Spring). 2016;24(5):1018–1026.
3. Waterland RA, Michels KB. Epigenetic epidemiology of the developmental origin hypothesis. Ann Rev. Nutrition. 2007;27:363–388.
4. Hayward CE, Lean S, Sibely CP, Jones RL, Wareing M, Greenwood SL. Placental adaptation: What can we learn from birthweight: placental weight ratio? Front. Physio. 2016;3(1):728.
5. Vaughan OR, Fowden AL, Coan PM. Morphological adaptations of the mouse placenta with maternal undernutrition. Proc. Physio. Soc. 2008;11:38.
6. Leon DA, Lithell HO, Vågerö, D. Reduced fetal growth rate and increased risk of death from ischemic heart disease: cohort study of 15,000 Swedish men and women born 1915–29. BMJ. 1998;317(7153): 241–245.
7. Boney CM, Verma A, Tucker R, Vohr BR. Metabolic syndrome in childhood: association with birth weight, maternal obesity, and gestational diabetes mellitus. J Pediatrics. 2005;115(3):290–296.
8. Harder T, Rodekamp E, Schellong K, Dudenhausen JW, Plagemann A. Birth weight and subsequent risk of type 2 diabetes: a meta-analysis. Am J Epidem. 2007;165(8):849–857.
9. Roos S, Lagerlöf O, Wennergren M, Powell TL, Jansson T. Regulation of amino acid transporters by glucose and growth factors in cultured primary human trophoblast cells is mediated by mtor signalling. AJP Cell Physio. 2009;297:723–731.
10. Zhang S, Regnault RH, Barker PL, Botting KJ, McMillen IC, McMillan CM et al. Placental Adaptations in Growth Restriction, Review. Nutrients. 2015;7(1):360-389.
11. Lager S, Powell TL. Regulation of nutrient transport across the placenta. Pregnancy. 2012;(7153):179827.
12. Fowden AL, Sferruzzi-Perri AN, Coan PM, Constância M, Burton GJ. Placental efficiency and adaptation: endocrine regulation. J Physio. 2009;587(14):3459–3472.
13. Myatt L. Placental adaptive responses and fetal programming. Physiology. 2006;572(Pt1):25-30.
14. Pal SK, Shukla Y. Herbal medicines: current status and the future. Asian Pacific J Cancer. 2003;4(4):281-288.
15. World Health Organization. Traditional Medicines strategy, 2002-2005. 2002.
16. Ekor M. The growing use of herbal medicines: issues relating to adverse reactions and challenges in monitoring safety. Front. Pharmacol. 2014;4:177.
17. Owolabi MS, Avoseh ON, Ogunwande IA, Setzer WN, Ogungbo R, Ogundajo AL et al. Chemical composition of Citrus limon (L.) Osbeck growing in southwestern Nigeria: Essential oil chemo types of both peel and leaf of lemon. Am J Essentials Oils and Natural products. 2018;6(4):36-40.
18. Aiyeloja AA, Bello OA. Ethno botanical potentials of common herbs in Nigeria: A case study of Enugu state. Educational Res. Rev. 2006;1(1):16-22.
19. Karacor K, Cam M. Effects of oleic acid on lipid profile. Med. Sci. Discov. 2015;2(1):125-132
20. Oguwike FN, Onubueze DP. Evaluation of efficacy of lemon juice extract (citrus lemon risso) on wound healing and haemostatic mechanism of albino Wister rats. IJSR. 2013;85:5.
21. Samundy K, Ranjana V, Biji B. Effect of warm lemon water drink on selected physical parameters among the overweight female nursing students of RIMS and RUP. Int J Nursing Res Pract. 2016;3(1):2350-1324.
22. Touhami M, Laroubi A, Elhabazi K, Loubna I, Zrara Y, Eljahiri A et al. Lemon juice has protective activity in rat's urolithiasis model. BMC Urology. 2007;7:1-10.
23. American College of Obstetricians and Gynecologists (ACOG). Morning Sickness: Nausea and Vomiting of Pregnancy. 2020
24. Lixandru M. Foods to eat for nausea: Nausea Remedies. NatureWord. 2016;1-6
25. NIH. Guide for the Care and Use of Laboratory Animals. NIH Publication revised 1996;85-23.
26. Laaksonen KS, Nevalainen TO, Haasio K, Kasanen IHE, Nieminen PA, Voipio HM *et al*. Food and water intake, growth and adiposity of Sprague-Dawley rats with diet board for 24 months. Laboratory Animals. 2013;47(4):245-256.
27. Khan Y, Rafeeq AK Syeda A, Afshan S. Evaluation of hypolipidemic effect of Citrus limon. J Basic and applied Sci. 2010;6(1):9-43.
28. NIST Standard Reference Database, NIST 08 MS Library and MS Search Programme v2.0f. 2008.
29. Pereda J, Gomez-Cambronero L, Alberola A, Fabregat G, Cerda M, Escober J, *et al*. Co-administration of pentoxifylline and thiopental causes death by acute pulmonary oedema in rats. British J Pharmacol. 2006;149(4):450-455.
30. Nascente LMP, Grandi C, Aragon DC, Cardoso VC. Placental measurements and their association with birth weight in a Brazillian cohort. Rev.bras.epidemiol. 2020; 23
31. Gallavan RH, Jr Holson JF, Stump DG, Knapp JF, Reynolds VL. Interpreting the toxicologic significance of alterations in anogenital distance: potential for confounding effects of progeny body weights. Repro Toxicol. 1999;13(5):383-390.
32. Muralimanoharan S, Maloyan A, Myatt L. Mitochondrial function and glucose metabolism in the placenta with gestational diabetes mellitus: role of miR-143. Clin Sci (Lond). 2016;130(11):931-941
33. Tamura RK, Sabbagha RE, Julien SD. Altered fetal growth. The Global Lib Women's Med. 2008;1027: 1756-2228.
34. Alur P. Sex Differences in Nutrition, Growth and Metabolism in Preterm Infants Front Pediatrics. 2019;7:22.
35. Stark MJ, Clifton VL, Wright IM. Neonates born to mothers with preeclampsia exhibit sex-specific alterations in microvascular function. Pediatric Res. 2009; 65(3):292–5
36. Clifton VL. Review: sex and the human placenta: mediating differential strategies of fetal growth and survival. Placenta. 2010;31(Suppl):S33–9.
37. Miwa I, Sase M, Torii M, Sanai H, Nakamura Y, Ueda K *et al*. A thick placenta: a predictor of adverse pregnancy outcomes, Springerplus. 2014;3:353.

38. Fox H. Pathology of the placenta. Philadelphia: WB Saunders; 1978. p. 355-356.
39. Raio L, Ghezzi F, Cromi A, Nelle M, Durig P, Schneider H *et al*. The thick heterogeneous (jellylike) placenta: a strong predictor of adverse pregnancy outcome. Prenatal Diagnosis. 2004;24:182-188.
40. Brett KE, Ferraro ZM, Yockell-Lelievre J, Gruslin A, Adamo KB. Maternal–Fetal Nutrient Transport in Pregnancy Pathologies: The Role of the Placenta. Intl J Mol Sci. 2014;15(9):16153–16185.
41. Gude NM, Roberts CT, Kalionis B, King RG. Growth and function of the normal human placenta, Thrombosis Res J. 2004;114(5-6):397-407.
42. Tan EK, Tan EL. Alterations in physiology and anatomy during pregnancy. Best Pract Res Clin Obst Gynaecol. 2013;27(6):791-802.
43. Stuat BC, Galan HL, Harwood JE, Lee G, Marconi AM, Paolini CL et al. Transplacental supply of mannose and inositol in uncomplicated pregnancies using stable isotopes. J Clin Endocrinol Metab. 2012;97:2497–2502.
44. Larque E, Ruiz-Palacios M, Koletzko B. Placental regulation of fetal nutrient supply. Curr Opin Clin Nutri Metab Care. 2013;16:292–297.
45. Gauster M, Hiden U, Blaschitz A, Frank S, Lang U, Alvino G *et al*. Dysregulation of placental endothelial lipase and lipoprotein lipase in intrauterine growth-restricted pregnancies. J Clin Endocrinol Metab. 2007;92:2256–2263.
46. Schaiff WT, Bildirici I, Cheong M, Chern PL, Nelson DM, Sadovsky Y *et al*. Peroxisome proliferator-activated receptor-gamma and retinoid X receptor signalling regulates fatty acid uptake by primary human placental trophoblasts. J Clin Endocrinol Metab. 2005;90:4267–4275.
47. Avagliano L, Garo C, Marconi AM. Placental amino acids transport in intrauterine growth restriction. Pregnancy. 2012;972562:1-6.
48. Cramer S, Beveridge M, Kilberg M, Novak D. Physiological importance of system A mediated amino acid transport to rat fetal development. AJP Cell Physiology. 2002;282(1):C153-60.
49. Salavati N, Gordijn SJ, Sovio U, Charles AK, Erwich JJ, Plösch T et al. Birth weight to placenta weight ratio and its relationship to ultrasonic measurements, maternal and neonatal morbidity: A prospective cohort study of nulliparous women. Placenta. 2018;63:45-52