Physiology and Pharmacology 26 (2022) 459-467
Original Article

Effect of prenatal consumption of *Combretum dolichopetalum* by pregnant rats on haematological and biochemical parameters as well as pregnancy outcome

Chinedum U. Emelike[1*], Ugochukwu S. B. Anyaehie[2], Eghosa E. Iyare[2], Chiemeziem A. Obike[3], Chinyere Aloke[4,8], Ofovwe O. Ekakitie[1], John A. O. Chukwu[5], Ndukwe Maduka[6], Odochi O. Chukwu[1,7]

1. Department of Physiology, Faculty of Basic Medical Sciences, College of Medical Sciences, Alex Ekwueme Federal University, Ndufu-Alike, Abakaliki, Ebonyi State, Nigeria
2. Department of Physiology, Faculty of Basic Medical Sciences, College of Medicine, University of Nigeria, Enugu Campus, Enugu, Nigeria
3. Department of Biochemistry, Michael Okpara University of Agriculture Umudike, Umuahia, Abia State, Nigeria
4. Department of Medical Biochemistry, Faculty of Basic Medical Sciences, College of Medical Sciences, Alex Ekwueme Federal University, Ndufu-Alike, Abakaliki, Ebonyi State, Nigeria
5. Department of Physiology, Faculty of Basic Medical Sciences, College of Medicine, Ebonyi State University, Abakaliki, Ebonyi State, Nigeria
6. Department of Biological Sciences, College of Natural and Applied Sciences, Wellspring University, Benin City, Edo State, Nigeria
7. Physiology Unit, College of Health Sciences, Evangel University Akaeze, Ebonyi State, Nigeria
8. Protein Structure-Function and Research Unit, School of Molecular and Cell Biology, Faculty of Science, University of the Witwatersrand, Braamfontein 2050, Johannesburg, South Africa

ABSTRACT

Introduction: *Combretum dolichopetalum* (CD) is commonly found in the Eastern part of Nigeria where it is used to relieve menstrual pain, enhance labour, facilitate the removal of placenta and promote a rich milk supply after delivery. This study investigates the effect of prenatal consumption of *Combretum dolichopetalum* by pregnant albino rats on haematological, and biochemical parameters as well as pregnancy outcome.

Methods: Mature inbred healthy female albino rats of normal estrus cycles that were 2-3 months of age weighting 120-180 g were used for the study. Examination of the estrus cycle, the introduction of male rats at pro-estrus, and confirmation of pregnancy were adopted using standard method. After initiation of pregnancy, fifty (50) rats were placed in five groups comprising ten rats per group. Distilled water was administered to rats in Group 1 which served as control while rats in Groups 2, 3, 4, and 5 received 100, 200, 400 and 800 mg/kg of *Combretum dolichopetalum* methanol leaf extract (CDLE) from day 15 to 20 of pregnancy using oral gavage, respectively. Maternal weight, haematological parameters (full blood count), biochemical parameters (renal and liver indices), gestational length, and litter size were measured using standard methods.

Results: The result showed a decrease in maternal weight, postpartum weight retained and gestational length, an increase in haematological parameters, and no changes in the renal and liver indices.

Conclusion: This study indicates that CDLE during prenatal did not influence the pregnancy outcome but was beneficial in decreasing postpartum weight retained without any visible sign of toxicity.

Keywords:
Combretum dolichopetalum
Biochemical and Haematological parameters
Pregnancy outcome

* Corresponding author: Chinedum Uche Emelike, chinedum.emelike@funai.edu.ng
Received 13 February 2021; Revised from 6 June 2021; Accepted 16 October 2021

Citation: Emelike C.U., Anyaehie U.S.B., Iyare E.E., Obike C.A., Aloke C, Ekakitie O.O., Chukwu J.A.O., Maduka N, Chukwu O.O. Effect of prenatal consumption of *Combretum dolichopetalum* by pregnant rats on haematological and biochemical parameters as well as pregnancy outcome. Physiology and Pharmacology 2022; 26: 459-467. http://dx.doi.org/10.52547/phypha.27.1.1

Introduction

Pregnancy is the period from conception to birth. During this period, there are various physiological changes the woman undergoes to better accommodate the embryo or fetus (Emelike et al., 2018). Findings from recent studies have shown that complementary and alternative medicine (CAM) is increasingly used for maternal health (Glover et al., 2003; Gibson et al., 2004).

Administering herbal supplements during pregnancy and labour is associated with several effects such as nausea, vomiting, reflux, skin problems as well as nutritional, colds and respiratory illnesses (Maats and Crowther, 2002; Nordeng and Havnen, 2004). The choice of herbal supplements by women is because of the notion that the products are safer to use during pregnancy than other pharmaceutical products (Hollyer et al., 2002).

Combretum dolichopetalum (CD) is a useful plant in Africa. It is commonly found in the eastern part of Nigeria. This herb is used to relieve menstrual pain, enhance labour, facilitate the removal of the placenta after delivery, and promote a rich milk supply after delivery (Personal communication). We had earlier studied the acute and sub-acute toxicity of CD leaves in animals (Emelike et al., 2020). Despite the myriads of biological properties of CD which is commonly used in African ethnomedicine, there is a paucity of information in the literature on the scientific basis for ethnomedicinal uses of this plant, especially during pregnancy and postpartum outcome.

Going by the traditional practice of administering CD in humans during pregnancy which has been scientifically proven to be safe, in the present study, we investigated the effect of CD consumption by pregnant rats on pregnancy outcome, post-natal growth, as well as haematological and biochemical parameters in the offspring.

Materials and methods

Plant materials

Fresh looking and mature leaves of CD were located and plucked in 2017 from its natural habitat in Nsukka, Enugu. The plant samples were authenticated by Mr. C. J. Onyeukwu, a taxonomist of the Plant Science and Biotechnology Department of the University, and the voucher specimen (UNH No.49a) of the plant was deposited at the herbarium.

Preparation of extract

The leaves were washed and air dried at room temperature for 7 days. Then, the leaves were ground into a coarse powder using an electric blender (model ms-233, China). The process of obtaining an extract from 2 kg of the ground leaves lasted for 48 h using Soxhlet extraction method described by Jensen (2007). Thereafter, the extract was collected and dried at low temperature (40^{o}C) to obtain a pasty dark-green extract which was used for animal experiments.

Animal experiments

Fifty (50) mature inbred healthy virgin female rats were procured from the Animal House, Department of Physiology, University of Nigeria Enugu Campus. They were acclimatized to their feed (Vital feed®, Nigeria) and water (which they had access to *ad libitum*) for two weeks before the commencement of the experiment. The study was carried out from January 2017 to August 2018. Approval for the study protocol was given by the College of Medicine Research Ethics Committee of the University with protocol number 026/02/2017. Already established institutional guidelines and the National Institute of Health (NIH) guidelines on the use of experimental animals were adopted in this study.

Initiation of pregnancy

The estrus cycle of 50 mature inbred healthy virgin female rats was monitored by examining their vaginal smears with the aid of the light microscope daily and rats with two consecutive regular four-day estrus cycles were used for this study. At pro-estrus, male rats were brought to the female cages in the ratio of 1:2 to allow mating to happen. When spermatozoa were observed in the vaginal smear of the female rats on the following morning is proof that mating had successfully taken place, thus regarded as day 1 of pregnancy (Mallie and Boudzoumou, 1996).

Experimental procedure

The body weight of the rats in each group before pregnancy (pre-pregnancy weight) was determined and the values obtained were recorded to the nearest (g) using the same weighing scale mentioned below. After the initiation of pregnancy, the 50 rats were randomly divided into five groups (1-5) of ten rats per group. The animals in Group 1 were given distilled water which served as

the control. Groups 2, 3, 4, and 5 were administered 100, 200, 400, and 800mg/kg of *Combretum dolichopetalum* leaf extract (CDLE) from day 15 of pregnancy to day 20 of pregnancy respectively using oral gavage. The maternal weight at delivery (postpartum weight) was determined on the day of delivery and the postpartum weight retained was obtained by subtracting the pre-pregnancy weight from the postpartum weight. The gestational length and litter size were also determined. Thereafter, 2% sodium pentobarbital (75 mg/kg) was used to anesthetize the rats intraperitoneally. Venous blood was obtained via the orbital and poured into EDTA and plain tubes. The blood collected was used for haematological and biochemical parameters. The birth weight and length of the offspring in each group at delivery were determined. At seven days' interval, the birth weight and length of the offspring were determined until postnatal day (PND) 21 of the offspring. At weaning (PND 21), ten offspring were randomly selected from each group for the determination of body weight, haematological and biochemical parameters (liver and renal indices).

Body weights

The changes in the weights of the rats were recorded using a digital electronic weighing scale model number Scout Pro SP 401 (China).

Haematological parameters

Red blood cell count (RBC), packed cell volume (PCV), hemoglobin concentration (HGB), mean cell volume (MCV), mean cell hemoglobin (MCH), mean cell hemoglobin concentration (MCHC), white blood cell count (WBC), and platelet count (PLT) were assayed using Coulter® Ac-T 5Diff AL, (Beckman Coulter, Inc. Port Matilda, Pennsylvania, USA).

Liver function tests

The liver indices analyzed include total bilirubin, direct bilirubin, alanine aminotransferase (ALT), aspartate aminotransferase (AST), and alkaline phosphatase (ALP) using A25 Biosystem, Barcelona, Spain.

Kidney function tests

Electrolytes (sodium, potassium, chloride, and bicarbonate) were estimated with Easylyte® analyzer Medica Corporation, Bedford, USA while urea and creatinine were analyzed with A25 Biosystem, Barcelona, Spain.

Statistical analysis

The results were analyzed using GraphPad Prism (GraphPad ® Software, San Diego, CA, USA). One-way ANOVA following Tukey's post hoc test was used for data comparison. The results were expressed as mean ± standard error of the mean (SEM). At $P<0.05$, the values were considered to be significant.

Results

A comparison between rats in Groups 5 and 1 (control) revealed that the decrease in postpartum body weight experienced by the rats was significantly different ($P<0.05$). However, there was no significant difference ($P>0.05$) in the postpartum weight gain of rats in Groups 2, 3, and 4. The rats in Group 5 which received the highest dose (800 mg/kg) of CDLE showed a significant decrease ($P<0.05$) in postpartum weight gain and percentage rise in postpartum weight when compared with rats in Group 1 (Table 1).

Table 2 presents the effect of CDLE consumption during pregnancy on gestational length and litter size. The result showed that there was a significant decrease ($P<0.05$) in the mean gestational length of rats in Groups 4 and 5 when compared with rats in Group 1. However,

TABLE 1: Effect of consumption of CDLE on body weight of pregnant rats

	Pre-pregnancy weight (g)	Postpartum weight @ delivery (g)	Postpartum weight gain (g)	% Rise in Postpartum weight
Group 1	118.58±2.10	169.10±2.64	50.52±2.09	42.70±2.75
Group 2	118.30±3.44	166.10±2.27	47.80±2.48	40.74±2.94
Group 3	117.58±2.15	165.20±2.86	47.62±2.51	40.40±2.29
Group 4	120.76±3.62	162.00±2.79	41.24±3.21	34.79±3.56
Group 5	117.96±2.42	151.12±2.76*	33.16±1.06*	28.28±1.08*

Values are mean ± SEM, n=10, *$P<0.05$ versus control. Group 1= normal control (pregnant rats + distilled water), Group 2= pregnant rats + 100 mg/kg CDLE, Group 3= pregnant rats + 200 mg/kg CDLE, Group 4 = pregnant rats + 400 mg/kg CDLE, Group 5= pregnant rats + 800 mg/kg CDLE, CDLE= *Combretum dolichopetalum* leaf extract.

TABLE 2: Effect of consumption of CDLE during pregnancy on gestational length and litter size

	Gestational length (days)	Number of pups
Group 1	21.50±0.20	6.00±0.71
Group 2	21.40±0.32	6.00±0.71
Group 3	21.00±0.32	6.00±0.37
Group 4	20.00±0.35*	5.00±0.32
Group 5	20.00±0.32*	6.00±0.37

Values are mean ± SEM, n=10, *P< 0.05 versus control. Group 1= normal control (pregnant rats + distilled water), Group 2= pregnant rats + 100 mg/kg CDLE, Group 3= pregnant rats + 200 mg/kg CDLE, Group 4 = pregnant rats + 400 mg/kg CDLE, Group 5= pregnant rats + 800 mg/kg CDLE, CDLE= *Combretum dolichopetalum* leaf extract.

TABLE 3: Effect of consumption of CDLE during pregnancy on haematological parameters of the dam

	Group 1	Group 2	Group 3	Group 4	Group 5
WBC X 10^9/L	9.36±0.91	9.70±0.36	9.82±0.32	10.20±0.40	10.36±0.53
N %	20.20±0.66	22.20±0.66*	25.60±0.96*	29.40±0.51*	33.83±0.47*
L %	77.40±0.75	74.36±0.45*	70.00±0.23*	66.00±0.19*	61.60±0.93*
M %	2.40±0.60	3.80±0.20	4.40±0.25*	4.60±0.40*	5..00±0.45*
RBC X 10^{12}/L	7.73±0.07	7.94±0.02	8.28±0.04	8.64±0.17*	9.20±0.30*
PCV (L/L)	39.02±0.00	39.93±0.13*	40.70±0.15*	41.07±0.14*	41.88±0.33*
HGB (g/dL)	13.00±0.09	13.31±0.13*	13.57±0.05*	13.69±0.04*	13.95±0.11*
MCV (fL)	46.12±0.24	47.11±0.06*	47.27±0.04*	47.40±0.09*	47.16±0.17*
MCH (pg)	17.10±0.08	17.26±0.02	17.39±0.05*	17.43±0.04*	17.60±0.15*
MCHC (g/dl)	35.26±0.04	35.68±0.12	35.96±0.13	35.48±0.39	35.78±0.21
PLT X 109/L	624.20±5.24	627.60±6.12	630.60±5.33	632.42±5.22	633.46±6.94

Values are mean ± SEM, n=10, *P< 0.05 versus control. NOTE: WBC-White blood cells; RBC-Red blood cells (x10^{12}/L); PCV-Packed cell volume (L/L); HGB-Hemoglobin concentration (g/dL); MCV-Mean cell volume (fL); MCH-Mean cell hemoglobin (pg); MCHC- Mean cell hemoglobin concentration (g/dL); PLT-Platelet count (x10^9/L). Group 1= normal control (pregnant rats + distilled water), Group 2= pregnant rats + 100 mg/kg CDLE, Group 3= pregnant rats + 200 mg/kg CDLE, Group 4 = pregnant rats + 400 mg/kg CDLE, Group 5= pregnant rats + 800 mg/kg CDLE, CDLE= *Combretum dolichopetalum* leaf extract.

there was no significant increase (P>0.05) in Groups 2 and 3 (Table 2). Similarly, there is no significant difference (P>0.05) in the litter size observed in all the test groups when compared with the control (Table 2).

The effect of administering CDLE during pregnancy on the haematological parameters of the dam is shown in Table 3. From the results obtained, there were significant increases (P<0.05) in the neutrophils, PCV, HGB, and MCV levels of rats in Groups 2 to 5 relative to those in Group 1. The results also showed that there was a significant decrease (P<0.05) in the level of lymphocytes of rats in Groups 2, 3, 4, and 5 when compared with that of Group 1. There were significant increases (P<0.05) in the level of monocytes of rats in groups 3 to 5 compared with rats in Group 1. There were significant increases (P<0.05) in the RBC counts of groups 4 and 5 rats compared with that of Group 1. There were no significant differences (P>0.05) in the WBC, MCHC, and PLT of rats in Groups 2 to 5 when compared with the control (Table 3).

Table 4 shows the effect of administering CDLE during pregnancy on renal indices of the dam. Accordingly, there were no significant differences (P>0.05) in the serum levels of Na^+, K^+, Cl^-, HCO_3^-, urea, and creatinine in Groups 2 to 5 rats when compared with the control.

The effect of administering CDLE during pregnancy on liver indices of the dam is shown in Table 5. The result presented shows that there were no significant differences (P>0.05) in the serum levels of total and direct bilirubin and the activities of ALT, AST, and ALP in the sera of Groups 2 to 5 rats compared with that of Group 1.

Table 6 represents the effect of rat body weight and body mass index at birth which significantly decreased (P<0.05) in Groups 4 and 5 in comparison with rats in

TABLE 4: Effect of consumption of CDLE during pregnancy on renal indices of the dam

	Group 1	Group 2	Group 3	Group 4	Group 5
Sodium (mEq/L)	140.28±0.18	140.31±0.12	140.62±0.11	140.70±0.12	140.85±0.11
Potassium (mEq/L)	4.00±0.10	4.10±0.76	4.11±0.00	4.12±0.26	4.13±0.70
Chloride (mEq/L)	100.28±0.10	100.47±0.16	100.76±0.10	100.81±0.25	101.10±0.26
Bicarbonate (mEq/L)	15.58±1.81	15.82±2.05	16.86±2.01	16.92±2.26	17.46±2.36
Urea (mg/dL)	6.31±0.66	6.33±0.47	6.74±0.10	6.83±0.47	7.12±0.54
Creatinine (mg/dL)	1.40±0.2.33	1.44±0.28	1.47±0.33	1.54±0.25	1.61±0.78

Values are mean ± SEM, n=10, $^{*}P< 0.05$ versus control. Group 1= normal control (pregnant rats + distilled water), Group 2= pregnant rats + 100 mg/kg CDLE, Group 3= pregnant rats + 200 mg/kg CDLE, Group 4 = pregnant rats + 400 mg/kg CDLE, Group 5= pregnant rats + 800 mg/kg CDLE, CDLE= *Combretum dolichopetalum* leaf extract.

TABLE 5: Effect of consumption of CDLE during pregnancy on liver indices of the dam

	Group 1	Group 2	Group 3	Group 4	Group 5
Total bilirubin (mg/dL)	5.00±0.13	5.13±0.25	5.24±0.36	5.31±0.19	5.41±0.82
Conjugated bilirubin (mg/dL)	2.36±0.34	2.28±0.30	2.26±0.41	2.28±0.31	2.33±0.33
ALT (U/L)	16.02±2.61	15.77±2.37	16.66±2.49	15.78±2.44	15.06±2.77
AST (U/L)	25.06±4.20	25.77±4.85	24.44±4.76	24.64±4.17	24.48±4.29
ALP (U/L)	196.22±0.01	196.77±0.66	196.88±0.99	197.18±0.80	197.36±0.27

Values are mean ± SEM, n=10, *= p< 0.05 versus control. NOTE: ALT-Alanine aminotransferase, AST-Aspartate aminotransferase, ALP-Alkaline phosphatase. Group 1= normal control (pregnant rats + distilled water), Group 2= pregnant rats + 100 mg/kg CDLE, Group 3= pregnant rats + 200 mg/kg CDLE, Group 4 = pregnant rats + 400 mg/kg CDLE, Group 5= pregnant rats + 800 mg/kg CDLE, CDLE= *Combretum dolichopetalum* leaf extract.

TABLE 6: Effect of consumption of CDLE on body weight, length, and body mass index of the offspring at birth

	Body weight @ birth (g)	Length @ birth (cm)	Body mass index@ birth (kg/m^2)
Group 1	4.83±0.09	5.15±0.09	1.83±0.08
Group 2	4.76±0.10	5.07±0.09	1.86±0.08
Group 3	4.62±0.10	5.01±0.10	1.85±0.12
Group 4	4.16±0.10*	5.01±0.10	1.12±0.02*
Group 5	3.83±0.10*	4.15±0.08*	1.52±0.01*

Values are mean ± SEM, n=10, $^{*}P< 0.05$ versus control. Group 1= normal control (pregnant rats + distilled water), Group 2= pregnant rat + 100 mg/kg CDLE, Group 3= pregnant rat + 200 mg/kg CDLE, Group 4 = pregnant rat + 400 mg/kg CDLE, Group 5= pregnant rat + 800 mg/kg CDLE, CDLE= *Combretum dolichopetalum* leaf extract.

Group 1. Our results show that there was no significant difference ($P>0.05$) in the body weight of the rats at birth in Groups 2 and 3 (Table 6). On the other hand, rats in Group 5 which received the highest dose (800 mg/kg) of CDLE showed a significant decrease ($P<0.05$) in body length when compared with rats in Group 1. The body length of rats in Groups 2 to 4 was not significantly different ($P>0.05$) from that of the control group.

The effect of administering CDLE during pregnancy on the haematological parameters of the offspring at weaning is shown in Table 7. The results presented showed that there were significant increases ($P<0.05$) in the WBC, neutrophils, RBC, PCV, HGB, and MCV levels of Groups 4 and 5 rats relative to that of Group 1. The results obtained indicated that there was a significant decrease ($P<0.05$) in lymphocytes of rats in Groups 4 and 5 when compared with that of Group 1. However, there were no significant differences ($P>0.05$) in the level of monocytes, MCHC, and PLT of rats in Groups 2 to 5 in comparison to the control (Table 7).

Table 8 shows the effect of administering CDLE during pregnancy on renal indices of the offspring at weaning. Our results show that there were no significant differences ($P>0.05$) in the serum levels of Na^+, K^+, Cl^-, HCO_3^-, urea, and creatinine in Groups 2 to 5 rats when compared with the control.

TABLE 7: Effect of consumption of CDLE during pregnancy on haematological parameters of the offspring at weaning

	Group 1	Group 2	Group 3	Group 4	Group 5
WBC X 10^9/L	10.16±0.22	10.18±0.33	10.66±0.42	12.80±0.65*	13.75±0.57*
Neutrophils (%)	20.20±0.66	21.00±0.33	21.60±0.51	27.50±0.51*	29.00±1.18*
Lymphocytes (%)	77.40±0.85	76.80±0.85	76.00±0.73	70.00±0.94*	68.10±0.93*
Monocytes (%)	1.90±0.88	2.10±0.10	2.40±0.15	2.50±0.30	2.60±0.25
RBC X 1012/L	7.33±0.57	7.44±0.51	7.56±0.23	8.44±0.12*	8.56±1.15*
PCV (L/L)	41.02±0.20	41.10±0.33	41.30±0.22	42.90±0.44*	43.36±0.39*
HGB (g/dL)	13.65±0.12	13.71±0.12	13.76±0.11	14.32±0.12*	14.47±0.10*
MCV (fL)	46.12±0.17	46.22±0.24	46.34±0.25	49.31±0.33*	49.55±0.22*
MCH (pg)	17.10±0.08	17.16±0.02	17.20±0.05	18.33±0.12*	18.44±0.11*
MCHC (g/dl)	36.76±0.24	36.87±0.32	36.96±0.33	37.09±0.11	37.12±0.10
PLT X 10^9/L	674.10±3.65	674.20±4.22	675.20±4.11	676.20±4.33	677.40±4.54

Values are mean ± SEM, n=10, *P< 0.05 versus control. NOTE: WBC-White blood cells; RBC-Red blood cells (x10^{12}/L); PCV-Packed cell volume (L/L); HGB-Hemoglobin concentration (g/dL); MCV-Mean cell volume (fL); MCH-Mean cell hemoglobin (pg); MCHC- Mean cell hemoglobin concentration (g/dL); PLT-Platelet count (x10^9/L). Group 1= normal control (pregnant rats + distilled water), Group 2= pregnant rats + 100 mg/kg CDLE, Group 3= pregnant rats + 200 mg/kg CDLE, Group 4 = pregnant rats + 400 mg/kg CDLE, Group 5= pregnant rats + 800 mg/kg CDLE, CDLE= *Combretum dolichopetalum* leaf extract.

TABLE 8: Effect of consumption of CDLE during pregnancy on renal indices of offspring at weaning

	Group 1	Group 2	Group 3	Group 4	Group 5
Sodium (mEq/L)	140.41±0.62	140.53±0.10	140.69±0.71	140.78±0.44	140.98±0.12
Potassium (mEq/L)	4.01±0.15	4.02±0.14	4.04±0.13	4.06±0.12	4.08±0.11
Chloride (mEq/L)	100.28±0.37	100.31±0.25	100.48±0.78	100.71±0.77	101.80±0.81
Bicarbonate (mEq/L)	16.58±0.99	16.72±0.75	16.84±0.88	16.92±0.46	16.99±0.30
Urea (mg/dL)	7.20±0.33	6.82±0.22	7.14±0.30	7.22±0.47	7.32±0.54
Creatinine (mg/dL)	1.45±0.11	1.42±0.18	1.47±0.12	1.49±0.15	1.51±0.18

Values are mean ± SEM, n=10, *P< 0.05 versus control. Group 1= normal control (pregnant rats + distilled water), Group 2= pregnant rats + 100 mg/kg CDLE, Group 3= pregnant rats + 200 mg/kg CDLE, Group 4 = pregnant rats + 400 mg/kg CDLE, Group 5= pregnant rats + 800 mg/kg CDLE, CDLE= *Combretum dolichopetalum* leaf extract.

TABLE 9: Effect of consumption of CDLE during pregnancy on liver indices of offspring at weaning

	Group 1	Group 2	Group 3	Group 4	Group 5
Total bilirubin (mg/dL)	4.01±0.56	4.03±0.52	4.14±0.45	4.26±0.23	4.42±0.55
Conjugated bilirubin (mg/dL)	1.86±0.78	1.98±0.10	2.03±0.12	2.14±0.33	2.20±0.69
ALT (U/L)	15.02±1.63	15.12±1.77	15.26±1.49	15.38±1.33	15.56±1.89
AST (U/L)	25.02±1.20	25.52±1.65	25.34±1.56	25.24±1.71	25.18±1.69
ALP (U/L)	197.45±1.50	198.28±1.77	198.41±1.74	198.58±1.68	198.86±1.58

Values are mean ± SEM, n=10, *P<0.05 versus control. NOTE: ALT-Alanine aminotransferase, AST-Aspartate aminotransferase, ALP-Alkaline phosphatase. Group 1= normal control (pregnant rats + distilled water), Group 2= pregnant rats + 100 mg/kg CDLE, Group 3= pregnant rats + 200 mg/kg CDLE, Group 4 = pregnant rats + 400 mg/kg CDLE, Group 5= pregnant rats + 800 mg/kg CDLE, CDLE= *Combretum dolichopetalum* leaf extract.

The effect of administering CDLE during pregnancy on liver indices of the offspring at weaning is shown in Table 9. The results presented indicate that there were no significant differences (P>0.05) in the serum levels of total and direct bilirubin and the activities of ALT, AST, and ALP in the sera of Groups 2 to 5 rats compared with rats in Group 1.

Discussion

The relationship between the use of safe, effective, quality products and practices based on local available evidence of finished biological active botanical substances are limited and quantities of these active ingredients that are given to the dams during pregnancy may be transmitted to the offspring via placenta in-utero (Ajar-

em and Ahmad, 1998).

This study has shown that there was a decrease in the postpartum weight retained at delivery in the rats that consumed this extract during pregnancy. Furthermore, administration of the extract at 800 mg/kg led to a significant decrease in body weight. Oftentimes, pregnancy is associated with postpartum retention of weight and several studies have shown the relationship between pregnancy weight gain and postpartum weight retention (Kac et al., 2004; Iyare and Obaji, 2014). The higher the pregnancy weight gain, the higher the postpartum weight retained and vice versa (Iyare and Obaji, 2014). The decrease in postpartum weight retained could be a result of reduced feed intake during pregnancy. This effect could be attributed to p, α-dimethylphenethylamine (aptrol), and dextroamphetamine, which are phytocomponents of the extract that has been reported to have appetite depressants properties and used in the management of obesity, respectively (Emelike et al., 2021). Secondly, the presence of tannin and saponin in the leaf extract is associated with anti-nutritional and toxic effects which include reduced feed intake, growth rate, feed efficiency, net metabolizable energy, and lowering of cholesterol levels could be linked with the decrease in postpartum weight retained (Emelike et al., 2021).

The result revealed a change in the gestational length in Groups 4 and 5 (Table 2). The presence of calcium in the extract might have contributed to the uterine contractile activities induced by CDLE (Emelike et al., 2021). This indicates that CDLE had uterine contraction properties which could have attributed to the change in the gestational length of Groups 4 and 5 at a dose dependent. This study revealed that no change in the litter size in all the test groups occurred (Table 2). This indicates that CDLE when administered to pregnant rats at the dosage used for this study, had no adverse effect on the number of pups delivered.

It is well established that haematological values may be quite valuable in assessing the toxicity effects of substances as the adverse change in values of red blood cell parameters (Chernecky, 2003). This present study revealed a significant increase ($p<0.05$) in neutrophils, RBC, PCV, HGB, MCV, and MCH in rats fed with CDLE with the highest level of significance in Group 5 (800mg/kg). It is on record that most green leafy plants have been implicated in the elevation of RBC counts because of their high iron content and ability to improve bone marrow functions as a major site for erythropoiesis (Orhue et al., 2008; Saliu et al., 2012). The haemopoietic activity of CDLE could be attributed to the presence of flavonoids, manganese, zinc, and iron among others (Emelike et al., 2021). Though the mechanism is unknown, this process can be achieved through the stimulation of growth factors such as erythropoietin on the receptors of pluripotent progenitor stems cells in the bone marrow. This indicates that CDLE is likely to stimulate hematopoiesis and also act as an immune-erythropoietin stimulating agent.

No doubt, several drugs rapidly cross the placenta and pharmacologically significant concentrations equilibrate in maternal and fetal plasma. The increase in blood parameters of the offspring of rats in Groups 4 and 5 at weaning (day21), suggests that components of the extract have hematopoietic properties and also can cross the placenta. This has been confirmed by a work done by Schroder (1998) who reported that flavonoid crosses the placenta and enters the fetal tissues. This is also true for minerals and vitamins.

The proper levels of sodium and potassium are essential for normal cell function. Their functions are regulation of the heartbeat, function of the muscles, maintaining the normal distribution of water, and the osmotic pressure in the extracellular and intracellular fluid compartments (Scott et al., 1999; Cheesbrough, 2005). Sodium and potassium concentrations were not significantly affected by the various dose levels of CDLE (pregnant and their offspring) which further gives credence to its safety. Chloride is the major anion found in the extracellular fluid and the blood. It also plays a role in helping the body maintain a normal balance of fluids, osmotic pressure, and anion-cation balance in the extracellular fluid compartment (Fogh-Anderson et al., 1984). This suggests that the administration of CDLE to pregnant rats and their offspring was not toxic. The bicarbonate ions act as a buffer to maintain the normal level of acidity (pH) in blood and other fluids in the body. Disruptions in the normal bicarbonate level may be due to diseases that interfere with respiratory function, kidney diseases, metabolic conditions, or other causes (Tietz, 1996). This suggests that the administration of CDLE to pregnant rats and their offspring at the dosage used for this study had no adverse effect. Urea is formed in the liver and is mainly excreted by the kidneys. The kidney is the most important route of urea excretion and

as a result, urea has long been used as a barometer of renal function (Corbette, 2008). This study has shown that urea concentration was not significantly affected by the dose levels of CDLE (pregnant and their offspring) and further gives credence to its safety. Also shown in this study is that the concentration of creatinine was not significantly affected by the dose levels of CDLE (pregnant and their offspring) and further gives credence to its safety. Most creatinine originates from the non-enzymatic conversion of creatine in muscle and elevated values may be associated with muscular dystrophy and anemia (Shivaraj et al., 2010).

Bilirubin usually formed from the breakdown of erythrocytes could be useful in evaluating liver function or haemolysis. The plasma loosely bound in albumin then carries the bilirubin. In the bound form, it is not soluble in water (Shivaraj et al., 2010). Bilirubin concentration was not significantly affected by the dose levels of CDLE (pregnant rats and their offspring) and further gives credence to its safety. Liver enzymes aspartate aminotransferase (AST) and alanine aminotransferase (ALT) concentrations in the treated rats (pregnant rats and their offspring) were not significantly increased following the administration of CDLE when compared with control. Liver function tests provide useful information regarding the condition of the liver. In addition, it describes the functionality of the liver (albumin and lipid profile) and its cellular integrity (transaminases). The information obtained is linked with the biliary tract (ALP) (Ezejiofor et al., 2013). It has been reported that liver toxicity is associated with an increase in various serum liver enzymes resulting from damage to the hepatocytes. The levels of these enzymes in all treated rats remained within the safety range and suggest that the use of CDLE for medicinal purposes may be safe. Our findings revealed the presence of phytochemical substances which has medicinal value in CDLE. Some of the substances have been reported to exhibit hepatoprotective activity. It is interesting to mention the role of flavonoids (Kumar et al., 2013; Emelike et al., 2021).

Conclusion

This study has revealed some beneficial effects of CDLE during pregnancy which are; an increase in postpartum weight loss and haematological parameters, a decrease in postpartum weight retained and a decrease in gestational length, and no change in renal and liver indices. These benefits justify the use of CD extract by traditional health practitioners to enhance labour and remove of placenta after delivery. Therefore, the use of this extract during pregnancy is recommended.

Acknowledgments

The authors are grateful to the staff of the Department of Physiology, Faculty of Basic Medical Sciences, College of Medical Sciences, Alex Ekwueme Federal University, Ndufu-Alike, Abakaliki, Ebonyi State, Nigeria for providing the enabling environment to conduct the research.

Conflict of interest

The authors declare that they have no conflict of interests.

References

Ajarem JS, Ahmad M. Effects of perinatal exposure of mice to non-alcoholic malt beverage “beer” on their offspring. Saudi J Biol Sci 1998; 5: 78-92.

Cheesbrough M. District Laboratory Practice in Tropical Country part II, Second Edition Cambridge University press 2006; 299-320. https://doi.org/10.1017/CBO9780511543470

Chernecky CC, Krech RL, Berger BJ. Laboratory tests and diagonistic procedures. W.B Saunders Company, a division of Harcourt Brace & company, Philadelphia.2003; 252-255, 638-639.

Corbette JV. Laboratory tests and diagnostic procedures with nursing diagnoses. 2008; 90-107.

Emelike CU, Ezimah ACU, Anyaehie USB, Iyare EE, Obike CA and Emelike FO. Haematological profile and body mass index of pregnant women in Ndiagu-Echara, Ikwo, Ebonyi State. Proceedings of the FBMS Symposium of Faculty of Basic Medical Sciences, Federal University, Ndufu-Alike.2018; 1: 40-4.

Emelike CU, Anyaehie USB, IyareEE, Obike CA, Eleazu COand Chukwuma C.Acute and sub-acute toxicity studies on Combretum dolichopetalum Engl. & Diels leaves. Slov Vet Zb 2002; 57 (3): 105-14. https://doi.org/10.26873/SVR-899-2020

Emelike CU, Anyaehie USB, Iyare EE, Obike CA, Aloke, C, Chukwu, DF, Eleazu CO, Chukwu CJ, Ekakitie OO, Konyefom NG and Uzomba CG. Chemical Composition and Evaluation of Methanol Leaf Extract of Combretum dolichopetalum on Body Weights and Haematological Indi-

ces of Phenylhydrazine Induced-Anaemic Rats. Toxicol Int 2021; 28 (2): 135-44.

Ezejiofor CN, Orish CN, Orish EB. Effect of aqueous leaves extract of Costus afer on the liver and kidney of male albino wistar rats. Anc Science Life 2013; 33(1): 4-9. https://doi.org/10.4103/0257-7941.134554

Fogh-Anderson N, Wimberley PD, Thode J. Determination of sodium and potassium with ion-selective electrodes. Clin Chem 1984; 30:433-6. https://doi.org/10.1093/clinchem/30.3.433

Gibson PS, Powrie R and Star J. Herbal and alternative medicine use during pregnancy: a cross-sectional survey. Obstet Gynecol 2004; 97(4): 44-5 https://doi.org/10.1097/00006250-200104001-00108

Glover DD, Amonkar M, Rybeck BF, Tracy TS. Prescription, over the counter, and herbal medicine use in a rural, obstetric population. Am J Obstet Gynecol 2003; 188 (4): 1039-45. https://doi.org/10.1067/mob.2003.223

Hollyer T, Boon H, Georgousis A, Smith M, Einarson A. The use of CAM by women suffering from nausea and vomiting during pregnancy. BMC Complement Altern Med 2002; 2: 1-6. https://doi.org/10.1186/1472-6882-2-5

Iyare EE, Obaji NN. Effects of aqueous leaf extract of Azadira chtaindica on some haematological parameters and blood glucose level in female rats. Niger J Exp Clin Biosci 2014; 2: 54-8. https://doi.org/10.4103/2348-0149.135731

Jensen WB. The Origin of Soxhlex Extractor. J Chem Educ 2007; 84 (12):1913-14. https://doi.org/10.1021/ed084p1913

Kac G, Benicio MH, Velasquez-Melendez G, Valenta JG, Struchiner CJ. Gestational weight gain and prepregnancy weight influence postpartum weight retention in a cohort of Brazilian women. J Nutr Mar 2004; 134(3): 661-6. https://doi.org/10.1093/jn/134.3.661

Kumar M, Manish KG, Anit S, Goel RJ. Healing effects of Musa sapientum var. Paradisiacal in diabetic rats with co-occuring gastric ulcer, cytokines and growth factor by PCR amplification. BMC Complement Altern Med 2013; 13: 305. https://doi.org/10.1186/1472-6882-13-305

Maats F, Crowther C. Patterns of vitamin, mineral and herbal supplement use prior to and during pregnancy. Aust N Z J Obstet Gynaecol 2002; 42: 494-6. https://doi.org/10.1111/j.0004-8666.2002.00494.x

Mallie JP, Boudzoumou P. Functional Renal maturation in rats' neonates after prenatal exposure to furosemide. Pediatr Nephrol 1996; 10: 458-60. https://doi.org/10.1007/s004670050139

Nordeng H, Havnen G. Use of herbal drugs in pregnancy: a survey among 400 Norwegian women. Pharmacoepidemiol Drug Saf 2004; 13: 371-80. https://doi.org/10.1002/pds.945

Orhue ES, Idu M, Ataman JE, Ebite LE. Haematological and histopathological studies of Jatropha tanjorensis leaves in Rabbits. Asian J Biol Sci 2008; 1(2): 84-9. https://doi.org/10.3923/ajbs.2008.84.89

Saliu JA, Elekofehinti OO, Komolafe K, Oboh G. Effects of some green leafy vegetables on the haematological parameters of diabetic rats. J Nat Prod Plant Resour 2012; 2(4): 482-5.

Schroder-Van DJP, Vad Der HD, Rokos H, Mareales DG, Kohne J. Synthetic flavonoids cross the plancenta in the rat and are found in the brain. Am J Physiol Endocrinol Metab 1998; 274: 253-6. https://doi.org/10.1152/ajpendo.1998.274.2.E253

Scott MG, Heusel JW, LeGrys VA, Sigaard-Anderson O. Electrolytes and blood gases [blood gases and pH]. In: Burtis CA, Ashwood ER, eds. Tietz textbook of clinical chemistry, 3rd ed. Philadelphia: WB Saunders.1999; 1072-1088.

Shivaraj G, Prakash BD, Shruthi SK, Vinayak VH, Avinash AK, Sonai NV. Markers of renal function test. N. Am. J Med Sci 2010;2(4): 170-3.

Tietz NW. Biochemical assessment of liver function fundamentals of clinical chemistry 4th Edition card A, B, Edward R. A, W. B., Sanders C.1996; 31: 543-8.