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Assessment of Several Concentrations of Aqueous Extract of *Croton Lobactus*(Linn) on Reproductive Parameters of Wistar Rats

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ABSTRACT

The plant, *Croton lobatus* (*Euphobaceae*) is grown for many purposes, it is used as a remedy for women with reproductive issues. The goal of this experiment is to verify the consequence of *C. lobatus* on some reproductive parameters and leitters of matured female wistar rats. Eighty female rats were separated into 16 groups of 5 rats each and another group containing 5 male rats was used for mating the female rats. Group 1 received distilled water; other groups received 200, 400 and 1000 mg/kg/ wt/day orally for a period of 21 days respectively. During the 21 days of administration, the body weight and vaginal smear were taken and recorded every seven days and every day respectively, only animals on pro-estrus phase were either anesthetized or mated. Fertility parameters such as pregnancy outcomes, leuthenizing hormones (LH), follicle stimulating hormones (FSH) and Estrogen-2hormones (E-2), were observed and recorded. The results obtained revealed a considerable rise in body weight in all extract treated groups when linked with the control, with a significant increase in weight of generative organs which were dose dependent. Hormonal levels at the estrus phase were observed to be significantly elevated in LH, E-2 and FSH. Hematological assessments of gravid rats were done using standard procedures. The study revealed that the plant extract was safe at 200, 400 and 1000 mg/kg/wt. The hematological studies, showed a significant increase in the number of white and red blood cells. The gross examination of leitters revealed that the plant is safe in pregnancy, indicating that *C. lobactus* leaves favoured reproductive parameters.

1. Introduction

Croton lobactus is of the Crotonoideae family comprising 1300 species [1]. The plant possesses some biological properties as well as ethno medicinal uses, which were revealed from the works of [2]. The plant was active as antimicrobial [3], anti-parasitic agents and anticancer agents. The traditional uses include anti plasmodial, antibacterial [3], antifungal and pregnancy problems, anti-inflammatory, antioxidant, antidepressant, eye disease, purgative, antifungal, the root is used for treatment of threatened abortion and hiccups [4, 5, 6, 7]. The plant contained various phyto-constituents such as phenol, alkaliod, phytosterol, phenol, Flavonoids, tannins and phlobatannins which convey all biological activities of the plant [7]. Several communities in Africa, Asia and South America have utilized medicinal plants for the treatment of diseases for many years. The substances of plant origin have been useful in treating various illnesses including infections. Microorganisms usually bacteria may be found in nearly everywhere having the tendency to adapt

rapidly to the immediate environment. Infectious bacteria are accountable for substantial mortality and morbidity worldwide principally in developing countries owing to poor hygiene and congested living environment. [8]. Presently, studies on herbal medicines appear under different names, such as, phytomedicines, plant medicines and natural products all these referring to products processed from living organisms like: plants, marine organisms, animals, insects and microorganisms. Atropine and others like morphine, quinine, ephedrine, warfarin, salicin, digoxin, taxol, and hyosine are derivatives of extracts from conventional plants presently used in recent medicines. Result from ethno-botanical and ethno-medicinal studies have revealed relationship between medicinal use and laboratory results. Natural sources have become the starting points for most pharmacological agents [9].

2. Materials and Method

2.1. Sample Collection

Fresh leaves of *Croton lobactus* was collected from Eyean Community in Edo State, Nigeria and authenticated by the Department of Plant Biology and Biotechnology, Faculty of Life Sciences, University of Benin, Benin City, Edo State, samples. The leaves were collected and dried at room temperature, then put in an oven at 40 ^{0}C for 48 hours and pulverized into fine powder, filtered with 30-mesh sieve and stored in an air tight container for further use.

2.2. Extraction of Plant Materials

The 300 g of powdered plant material was extracted with 2000 ml of distilled water using kedjah heating mantle with 3000 ml beaker. The resulting crude extract was filtered with a cheese cloth and concentrated in a water bath set at 40 ^{0}C for 48 hours (2 days).

2.3. Experimental Animals

Female and male albino rats weighing approximately 180-200g were obtained from Pharmacology animal house University of Benin, Benin City. These animals were also adapted and maintained in the animal house with standard laboratory conditions met and animals fed with pelletized feed and tap water *ad libitum.*

2.4. Drugs and Chemicals

Drugs procured and used in the experiment included Sabultamol (GlaxoSmith Kline), oxytocin injection (laborate pharmaceutical Indian) and aqueous extract of *Croton lobactus* Linn.

2.5. Mating Procedures

Female rats were mated with males in cages in a ratio of 1:1 at pro-estrus. Mated females were separated from males and examined for the presence of sperm in their vagina which was considered as day zero of pregnancy.

2.6. Pregnancy out comes

The 72 Dams were divided into groups 1a – 6a and 1b– 6b making up a total of twelve groups and these dams were treated with 0 mg/kg/wt (1a and 1b), 0.5 iu oxytocin intraperitoneally (2a and 2b), 10 mg/kg sabultamol (3a and 3b) as controls and 200,400, 1000 mg\kg/wt orally of the plant extract (4a-6a and 4b-6b).The animals in all groups (1a-6a) were examined of uterinehorns for implantation or resumption sites, which was compared in terms of litter size while the animals in groups (1b-6b) were left to litter and the pups in this group were compared in terms of size and pup weight, mortality, day of appearance of furs and eye-opening day. All animals were examined

daily for signs of toxicity such as diarrheal, salivation, bleeding through virginal, tremors, yellowing of fur or fur erection and moribund or mortality for the period of 21 days, the rats was laparotomized under chloroform anaesthesia and sterile conditions. The uterus was examined and pregnancy result for the presence and position of resorption sites, live and dead foetuses. For the animals that were left to give birth, parturition day was noted. The number of live or dead pups was recorded, and their body mass was determined. Pups were examined for unpolished outer genetic abnormalities such as (clubfoot, anomalies of tail, open eyelids). Pup death rate up to 6 days (mortality), eye opening day and fur appearance were recorded. On the basis of these data, the indices were computed as follows : quantal gestation= (number of pregnant dams/number mated)x 100; implantation guide = (total number of implants/number mated) x 100; pre-implantation damage = [(number of corporalutea-number of implantations)/number of corporalutea) x 100; post-implantation damage =[(number of implantations - number of viable implantations)/number of implantations]x 100; viability guide = (number of viable pups on day 4 after delivery/number of live born pups) x 100; birth guide = (number of Pups born/number of implantations) x 100; foetal persistence ratio = (number of surviving pups/number of implantations) x 100; live birth guide = (number of live born pups/total number of pups born) x 100; gestation guide= (number of live pups/numbers of pregnant dams) x 100, and 100 mg/ml were also determined. The effect of sabultamol was also established.

2.7. Estrogenicity study

The female animals on attainment of three regular consecutive estrus cycles were divided randomly into four groups (A, B, C and D) with four rats each and treated as follows:
Control group (A) received purified water
Group B took150 mg/kg//wt/day aqueous leaves extract of *C. lobactus*
Group C took 500 mg/kg/day aqueous leaves extract of *C. lobactus*
Group D took1000 mg/kg/day aqueous leaves extract of *C. lobactus*
At the close of 21day periods, the underlisted parameters were evaluated:
Appearance of ovary after 21 days of treatment, nature of oocytes, production of secondary follicles, level of serum hormones of LH, FSH, E-2 cells and reproductive organ weight in relation to body weight.

2.8. Statistical analysis

The outcome from the studies was articulated as the mean ± SEM (standard error of mean). Statistical analysis was carried out using graph pad prism version 6, software (UK). One-way analysis of variance (ANOVA) was used to compare between the control and treated groups. $P < 0.05$, 0.01, 0.001 and 0.0001 indicated significant difference in all cases.

3. Results and Discussion

The bar chart below indicates an abortifacient effect of oxytocin, standard drug sabultamol and the pregnancy enhancing effect of different doses of extract on pregnancy outcome. The result shows that the extract was able to prevent preterm labor and abortion (Figure 1) showing G1 as normal control and G2 as negative control (oxytocin 0.5 iu) with high presence of resorption sites and G3 represent positive control (salbutamol 10 mg/kg) while G4, G5 and G6 represents the animals treated with the plant extract at doses of200, 400 and1000 mg/kg/wt. respectively

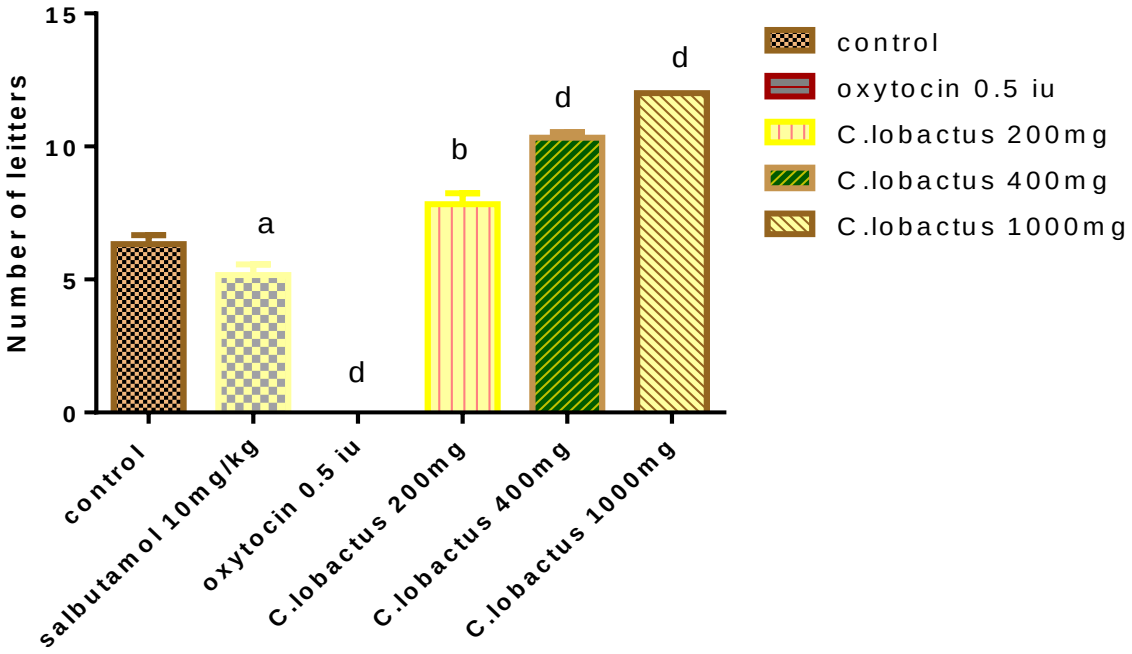

Figure 1: The effects of the extract on litters size in the uterus of the dams, the bar chat represents the control, positive control, Negative control, 200, 400 and 1000mg/kg of *C. lobactus* extract respectively.

Table 1 indicates various stages of pregnancy in rats with treated plant extract at varying concentrations when compared with negative and positive controls

Table 1: Out-come of aqueous extract of *Croton lobactus* leaves on Post implantation parameter.

Parameter	Control (N=5)	Nc (N =5)	Pc (N=5)	200,400,1000 mg/kg Mp (N=20)	150,500, 1000mg/kg Lp (N=20)
Nui	(6-8)	(0)	3-5	(5-10)(7-12)(10-12)	(5-10)(7-12)(10-12)
Qp(%)	100	000	100	100	100
Ii(%)	52.08	000	60	100	100
Pi (%)	4.3±0.5	100±0.^{b}	2.99±0.1^{a}	(1.75±3.6$^{a)}$)(1.5±0.4)(1.45±0.2)	(1.81^{a}±0.2)(1.3±0.5)(1±00)
Gi (%)	100	100	100	100	100
Mgd	21.1±0.2	21±00	21.2±0.2	(22.1±0.3^{a})(22.6±0.1)(23.±0.1^{b})	(23.4±0.4^{c})(24.1±0.1^{c})(25.3±01^{d})

Key: Qp = Quanta pregnancy, Nui = number of uterine implants, Mp = mid pregnancy, Nc = negative control, Pc = positive control, Mp = Mid-pregnancy, Lp = late pregnancy, G1 gestation index, Mgd= mean gestation days, Pi = post implantation.

The rats received 200, 400 and 1000 g/kg dailyfor21 days of gestation (mid to late pregnancy group, 21stto 26st day). Data are stated as means ±SEM*P<0.05,**P< 0.01 related to control using graph pad prism 6 Uk.

Table 2. Out-come of the aqueous extract of the leaves of *C. Lobactus* on litters of female rats.

Parameters	Npb(g)	Nlbp (mean)	Pwb(g)	Number of Deformed Pups	Birth index (%)	Pup Survival Ratio(%)	Life birth Index (%)	Eye opening day	Afd
Control	6.8±0.5	5.5±0.8	2.6±0.1	0.00	100	95.2±0.2	100	21±0.5	7.4±0.3
NC	5.5±0.8	1.6±1.1	2.8±0.3	0.00	100	35±0.1	100	21±0.3	9.1±0.1
200mg/kg	9.68±0.5[a]	6.8±0.3[a]	3.57±0.1	0.00	100	97.7±0.3[b]	100	14.9[b]	6.8±0.3[b]
400mg/kg	9.88±0.2[c]	9.7±0.3[c]	4.09±0.2[a]	0.00	100	98.7±0.3[c]	100	14±0.5[c]	6.3±0.4[c]
1000mg/kg	11.58±0.3[d]	12±0.0[d]	4.36±0.4[d]	0.00	100	100±0.2[d]	100	13.6±0.5[d]	6±0.1[d]

Key: Npb: Number of pups born, Nlbp = number of life born pups, Pwb = Pup weight at birth, Ndp = number of deform pup, Bi = birth index, psr = Pup survival ratio, Lbi = life birth index, Eod = eye opening day, Afd = appearance of fur day, NC= Negative control and PC = positive control, Afd = Appearance of fur day.

The gravid rats administered with 200, 400 and 1000 g/kg/wt of *C. lobatus* daily commencing from 0- 21 days and 10-26 days. Data were represented as means ±SEM a= P<0.05, b = P < 0.01, c = p<0.001 and 0.0001

Table 3 is a summary of the results obtained from hematological analysis of blood samples collected from the dams. The result reveals that the aqueous extract was found to have significantly increased white blood cell (WBC), Red blood cell (RBC), Pack cell volume(PCV), platelets (PLT), Neutrophil (NEU) and Basophil (BASO).

Table 3: Summary of the results obtained from hematological analysis of blood samples

Parameter mg/kg	WBC	RBC	PCV	PLT	NEU	BASO
Control	15.4±0.0	5.8±0.0	34.6±0.4	68.0±2.2	16.07±	1.24±0.1
200	16.2±0.2[a]	6.8±0.1[a]	36.5±0.1[b]	71.9±0.2	21.8±0.3[d]	1.40±0.0
400	17.3±0.1[d]	7.4±0.1[d]	39.6±0.4[d]	75.0±0.3[a]	21.4±0.1[d]	8.8±0.2[d]
1000	18.1±1.0[d]	8.0±0.1[d]	41.0±0.8[d]	76.0±0.6[d]	23.2±0.1[d]	8.44±0.1[d]

Key: [a]p< (0.05), [b]p< (0.01),[c] p< (0.001) and [d]p< (0.0001).

Results obtained from the body weight index of the dams during the course of pregnancy, revealed that even in the presence of induction of abortion by administration of oxytocin on day 12 the extract treated dams had successful progression of pregnancy and development (Table 4).

Table 4: Body weight index of the dams during the course of pregnancy

Treatment	Day0	Day7	Day14	Day21
Control	183.38±0.1	183.9±0.2	184.2±0.3	198±0.3[b]
Nc	187.8±04	188.6± 0.1	170±0.2	157.1±1.1
Pc	180±0.1	186±0.3[b]	186.1±0.5	187±0.2[c]
200mg/kg	187.8±3.3	192.1±3.9	195.7±2.4[a]	259.6±0.9[b]
400mg/kg	183.8±1.3	204.2±5.2	275.9±4.1[d]	285.1±3.8[d]
1000mg/kg	183±3.5	196.6±5.2[a]	207.6±2.6[c]	215.3±1.4[d]

Key: [a] p< (0.05)[b] p (0.01) [c] p< (0.001), and [d] p< (0.0001).

Table 5 shows the body mass and reproductive organ system mass connected by means of daily administration of *C. lobactus* extract to mature female rats after 21 days' treatment.

Table 5: Body mass and reproductive organ system mass after 21 days' treatment

Parameter	Control	200mg/kg	400mg/kg	1000mg/kg
Body weight(g)	184.5±0.6	188.5±0.22[a]	196.08±0.3[d]	200.7±0.4[d]
Reproductive organ weight(mg)	18.18[b]±0.4	20.7±0.5[b]	20.8[c]±0.3	22.1[d]±0.9

Key: [a] p< (0.05),[b]p (0.01) [c] p< (0.001) and [d] p< (0.0001).

Table 6: Hormonal change connected with daily administration of *C. lobactus* to mature female rats.

Hormone	Control	200mg/kg	400mg/kg	1000mg/kg
FSH (mlu/m)	4.08±0.2	5.00[a]±0.2	4.70±0.2	5.20[c]±0.1
LH (mlu/m)	3.06±0.2	5.54±0.5	7.00[a]±0.1	8.4[d]±0.2
EH (Pg/ml)	6.7±0.5	12.60[b]±1.4	14.4[c]±0.5	14.2±0.1

Key: FSH = follicle stimulating hormone (mlu/m), LH = Leuthenizing hormone (mlu/m), EH: Estrogen hormone **(**Pg/ml).

Standards are mean ± SEM. n = 6, [a]P<0.05 in contrast with the control group, [b]P< 0.01 [c]P< 0.001[d] P< 0.0001

Haematological outcomes indicated that there was increase in the count of white blood cells (WBC) at the different doses of the aqueous extract of *C. lobactus*, red blood cell counts (RBC) and packed cell volume (PCV) were improved, while other parameters such as lymphocyte

(LYM), eosinophil (EOS), basophil (BOS) counts and platelet aggregation (PLT) were observed to have been maintained unaffected. The effect of *C. lobactus* on the blood was dose dependent as the increase in count of the cells of the haemagenic series is a sign that the activation regulating the immune system have been boosted which correlates with the works of [10] on improved blood parameters. Information's concerning WBC counts pointed out platelet (PLT) linger unchanged, while amplified count of white blood cells (WBC) is expectedly oblige in boosting immune system as earlier reported by [10, 11]. Hence, a weighty increase in RBC with no change in PCV. Consequently, *C. lobactus* treated animals exposed to the extract causes no toxic consequence on RBC, red blood cell (RBC) and pack-cell volume (PCV) remains sign posted to be supportive in boosting immune system, calculations of haematological parameters are used to determine the level of decrease on the red blood cell (RBC) counts which was fortunately increased as the dose of the extract increases an indication that this plant is a good source of tonic, even the packed cell volume (PCV) count was improved as well suggestive of polycythemia and positive erythopoisis which correlates with the work of [12] but not in accordance with the work of [13], hence increase in RBC with no change in PCV. Therefore, *C. lobactus* treated animals exposed to the extract causes no toxic consequence on RBC. consequently, by reason of the positive effect of the extract, the platelets (PLT) aggregation was also increased, the lymphocyte (LYM) counts was also seen to increase, eosinophil (EOS) counts were improved and the counts of basophil (BOS) was increased, this also supports the histological findings as the results impressively propose that *C. lobactus* has potential effect as a naturally derived immune stimulant for the immune boosting this was supported with the work of [14]. Administration of *C. lobactus* to pregnant female rats induced body mass gains; this weight gain despite oxytocin administration at single dose level on day 12 revealed that the extract averted abortion and preterm labour. Weight loss was observed in the negative control group on administration of oxytocin at single dose level indicating abortion and preterm labour. Over the 21days of the experiment, body mass of negative control group was much less than positive control and extract treated groups. In contrast, rats administered with the extract of *C. lobactus* showed progressively increased in the body mass of the Dams at all the groups including the extract treated groups in which the weight increase was progressive as the dose increased, revealing that despite the induction of abortion with oxytocin and preterm labour on day twelve and day 21 of pregnancy there was progressive leitter development with the various doses of the extract which was an explanation of positive pregnancy outcome on the dams and their leitters.

The boost in body and reproductive organs mass may be due to the fact that the plants may be a good supply of tonic that arouse appetite reported in accordance to the work of [15], encourage body mass gain and acts as carminatives. The herb may have hypoglycaemic activity given that hypoglycemia is amongst the stimulators of growth hormone (GH) emission from the frontal pituitary gland backed by the report of [16]. The results from hormonal profile study showed rise in E-2 cells which can be ascribed to the fact that *C. lobactus* may possess some phytogenic property which may subsist energetically at little or much absorption and affects other target nerves and turns as agonist or antagonist to the E-2 hormones. Phyto-estrogens can deploy basic cell biology to their outcome on cellular enzymes. Increase in serum LH level after oral administration of the plants extract can be ascribed to high plasma concentrations of estrogen, as occur during the estrogen peak of the late follicular phase, which acts upon the pituitary to boost the sensitivity of LH discharging mechanism to GnRH. The high estrogen may also arouse further rise in the secretion of GnRH by the hypothalamus, although this remains contentious as sited by the work of [17]. Moreover, pre- ovulatory secretion of progesterone, although restricted, may exert a positive response on the estrogen-primed pituitary to enhance LH release. The study resulted in reduction in serum level of FSH in estrous phase of the estrous cycle; this may not affect ovulatory progression. This can be clarified as in the late follicular phase, FSH level decline in relation to the rise in E-2 levels which means that there is no need to increase FSH level in the

late follicular or ovulatory phase. At the same time, it has been observed that the dominant follicle existence (≥ 10 mm) is mainly LH – dependent. The negative response of E-2 is predominantly applied on FSH secretion at the pituitary level. Just before ovulation, after the GCs obtain LH receptors, LH also arouse the production of inhibin by these cells. The inhibin inhibits FSH production by the gonadotrophs confirming the work of [18]. After ovulation, the CL secretes E-2, progesterone and inhibin A under the control of LH this was in line with the work earlier done by [19]. The estrogens exert negative response at both low and high concentrations, while progestins are actual only at high concentration. These vivid events lead to decrease in FSH level. The results of *pregnancy* outcome disclose that *C. lobactus* may usefully manage pregnancy, prevent miscarriage as illustrated in Figure 1, negative control dams were discovered to possess high existence of resorption site this was not in consonance with [20]. As the negative control dams, which received oxytocin intraperitoneally on day twelve had no litters incidence but the high presence of resorption sites it was a clear sign of induced abortion by oxytocin in line with the citation of [21].

The litter size present in the uterine horns of the extract treated dams was found to be more in number and healthier when grossly compared with the normal control and those of the standard sabultamol (Figure 1) even in the presence of induced abortion and preterm labor on day twelve. Surprisingly, on sacrifice at day twenty-one the extract treated dams had extremely high number of litters presence up to ten (8-10 and 10-12) per dam Figure 1 this well correlated with [22].

From the results obtained from dams in the sub-group B left to leitter, the extract treated dams from day zero to twenty-one and at day twenty-one, oxytocin was given an hour after oral administration of various doses of the aqueous extract. It was observed that onset of labor was delayed for up to 73.3 hours, a genuine proof that the plant extract may have caused the delay in the onset of labor.

The pups delivered by the dams treated with the different doses of extract was observed to be grossly better in size, appearance and more in number and higher in weight this conformed with the work of [22]. The death rate of the pups arising from normal control, negative control and the positive control treated with sabultamol, the death rate was very high, as there were almost no survival records in 24 hours after their delivery. Meanwhile, for the extract treated dams there was a very high survival rates as there was almost no death records in 48 hours after parturition in line with the findings of [18]. Oral administration of the C. *lobactus* during mid and late pregnancy had positive effects on leitters in relations of uterine implants, gestation index or implantation index. Conversely, it posed no significant threat to fruitful pregnancy, as was considered by an increase in foetal existence ratio and birth index and reduction of post-implantation fatalities. These positive outcomes in pregnancy resulted from reduced developing deaths as obvious at laparotomy perhaps at mid stage of prenatal development since the leaves extract was non-toxic, and no correlation existed between maternal toxicity and growing toxicity, the use of the extract becomes embracing especially for women suffering incessant miscarriage and premature labour and are at high danger of miscarriage. The pups born by dams treated with the leaves extract at 400mg/kg and 1000 mg/kg had high mean birth weights. This indicated intra-uterine growth promotions was not owed to increase of gestational length without incidence of preterm delivery, and was probably no intrinsic deleterious effects as there was no negative foetal features such as chromosomal abnormalities or other malformations. However, it may be attributed to increased blood flow through the placental and essential supply of nutrients to the foetuses. The leaves extract appeared to be non-teratogenic in rodents. The viability index of pups born resulting leitters exposure to the aqueous leaves extract was high, even if some parameters of postnatal development such as day of eye opening and day of appearance of fur remained intact. If these facts can be applied to women, then eating of the leaves extract during pregnancy can be encouraged in countries like Nigeria where more than one third of all infants born are minor for gestational age. Normal birth weight has a key influence on neonatal wellbeing with less fear and

occurrence morbidity, neuro-cognitive deficiencies, with no negative neuro-behavioural effects and almost no mortality 200, 400 mg/kg with no mortality at 1000 mg/kg). Furthermore, increased growth in uterois is linked to foetal wellbeing and good foetal development.

4. Conclusion

Based on results obtained, it is therefore suggested that the usage of the *C. Lobactus* should be encouraged by pregnant women, since *C. lobactus* possess positive outcomes on pregnancy, prevents preterm labour, increased litter size and weight of Pups born was grossly better in appearance.

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