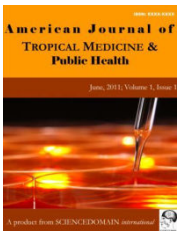

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Effect of Leaf Aqueous Extracts of *Vernonia amygdalina* Del on Contraction of Mammary Gland and Uterus of Guinea Pig Dams

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Research Article

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ABSTRACT

Aims: To determine the effects of aqueous extracts of *Vernonia amygdalina* on litter-weight gain, milk production and uterine contraction amplitude and mammary gland smooth muscle contraction amplitudes in the guinea pig model.
Methodology: The animals were placed in four groups of three animals each with group1 as control, and groups II-IV receiving 5 mg/kg, 10 mg/kg and 100 mg/kg *V. amygdalina* respectively. Using a chymograph, uterine and mammary gland contraction amplitudes were determined at 5, 10 and 100mg/ml of the extract with ergometrine as the control drug.
Results: After 5 weeks, the litter-weight of the control group (1.88 ± 0.68 g) was significantly ($P<0.05$) less than that in groups III (4.20 ±0.20 g) and IV (13.69 ± 1.53 g) but not different from group III (3.11 ± 0.10 g). All the dams had their highest mean milk production index (g) at week 2. From week 4, all the treatment groups produced more milk than the control group. At concentrations of 5 mg/ml and 10 mg/ml, uterine contraction amplitudes (mm) were 1.22 ± 0.03 and 3.60 ± 0.03 respectively. The contraction amplitude at 100 mg/ml matched that of ergometrine (9.58 ± 0.39 mm). The mammary gland contraction amplitudes (mm) recorded for 5 mg/ml, 10 mg/ml and 100 mg/ml *V. amygdalina* were 1.2 ± 0.14, 2.70 ± 0.36 and 6.15 ± 0.13 respectively. These values were significantly ($P< 0.05$) lower than those observed for the equivalent concentrations of ergometrine.
Conclusion: The results appear to support the claims of traditional birth attendants who use the plant to induce uterine motility and milk let-down after parturition.

Keywords: Vernonia amygdalina; mammary gland; uterus; milk production;

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1. INTRODUCTION

Vernonia amygdalina Del (Asteraceae) is a medium sized shrub used widely in Nigeria for both therapeutic and nutritional purposes (Ijeh and Ejike, 2011). It is found commonly in tropical West Africa and has a lot of bitter principles in every part of the plant which is due to anti-nutritional factors like alkaloids, saponins, tannins and glycosides (Bonsi et al., 1995).

The leaves of *Vernonia amygdalina* Del. has been shown to be useful as a remedy for gastrointestinal disorders and as a general tonic (Iwu, 1986). It has been shown to have blood sugar lowering effects in normal and alloxanized rabbits (Akah and Okafor, 1992), as well as high cholesterol lowering effects in diabetic rats (Uhuegbu and Ogbuehi, 2004). It has been shown to increase urinary and fecal output when included in the diet of mice (Igile et al., 1995). The plant showed protection against the toxic effects of aflatoxin B_1 exposure (Ijeh and Obidoa, 2004), and promise that it may help in kidney functions (Ijeh and Adedokun, 2006). A recent review by Ijeh and Ejike (2011) captures the medicinal properties of Vernonia amygdalina Del succinctly.

The present study was prompted by reports of previous workers (Kamatenesi-Mugisha, 2004) that feeding of *Vernonia amygdalina* produced uterine motility and increased the flow of milk after parturition in women in Uganda. The study is aimed at assessing the impact of a low, medium and high dose of the extract on the myoepithelial cells of the uterus and breast given that the extract is used at different concentrations that is usually not specified in ethnomedicinal preparations. Ijeh and Obidoa, (2004) and Akah and Okafor (1992) had independently reported the ED_{50} and LD_{50} respectively of aqueous extract of *Vernonia amygdalina.*

2. MATERIALS AND METHODS

2.1 Collection of Plants Materials

Fresh leaves of *Vernonia amygdalina* Del. were obtained from the University Demonstration Farm of Michael Okpara University of Agriculture, Umudike, Abia State, Nigeria. The plant samples were identified by Dr M.C. Dike of College of Natural Resources and Environmental Sciences, Michael Okpara University of Agriculture, Umudike. A specimen of the leaves was deposited in the Botany Department of the above University.

2.2 Preparation of Plant Extract

Freshly harvested leaves of *Vernonia amygdalina* Del. were dried under a fan at room temperature (20 - 25^{o}C) until a constant weight was achieved. The leaves were pulverized using a porcelain mortar and pestle to get a coarse powder used for the extraction. Fifty grams of the powdered leaves were macerated in 1L of distilled water for 48 hours with constant stirring before filtration and concentration *in vacuo* to yield 16.85g (11.23%). Doses of 5 mg/ml (low), 10 mg/ml (medium) and 100 mg/ml (high) in water were used.

2.3 Experimental Design

2.3.1 Animal treatment and grouping

Twelve female and four male guinea pigs were used for the study. They were acclimatized in the animal house for one week before beginning the experiments. The animals were

randomized according to their weights and housed in four stainless steel cages containing three females and one male each. They were exposed to 12hours light and dark cycles under humid tropical conditions.

Three females were grouped with each male for a period of 5 weeks until pregnancy was established in the females after which the males were separated from pregnant females.

2.3.2 Lactation studies

Each pregnant female was placed in a separate cage following parturition. They were administered with leaf extracts of the plant at doses of 5 mg/kg body weight (group II), 10 mg/kg body weight (group III) and 100 mg/kg body weight (group IV) intra-peritoneally. Female guinea pigs in group I served as control and received distilled water intra-peritoneally. Daily weight gain of litters was taken and dam weight was also taken in the morning (7-8am) before the litters suckled, and late afternoon (2-3pm) after the liters had suckled. The difference between the dam weight in the morning and afternoon was used as an index of milk production.

2.3.3 Mammary gland and uterine contraction studies

A modification of the David *et al.* (2000) method was used for these studies. Female guinea pigs weighing 250 - 350g were used in the uterine contraction experiments. The guinea pigs were humanely sacrificed. The abdomen was opened to expose the mammary tissue. The bilateral inguinal mammary glands were selected because they were the biggest. The cardiac and thoracic mammary glands were small and not selected. The muscle around the mammary area was cut at a point 5 – 10 mm below the skin and placed in a dish containing Ringer's solution. Great care was taken not to damage the mammary muscles. Portion of mammary muscle 1 - 2mm in length and free from mesenteric attachments were cut and tied at each ends. Each piece was then mounted in a tissue bath. Once mounted, each piece of the guinea pig mammary gland smooth muscle was allowed to stabilize for about 30 minutes until contractions became regular.

The uterus was also carefully dissected in a Petri dish containing Ringer's solution. Fat and connective tissues were removed. The horns of the uterus were then separated at the bifurcation, yielding two preparations. Each preparation was taken and mounted in the tissue bath for 30 minutes to normalize before adding the crude extract and standard drug (Ergometrine) so that spontaneous activity could be determined.

The plant extract or standard drug that was used for control was then injected separately into the tissue bath with the tissue and the kymograph recording machine connected to the slow running rotating drum and writing lever that was translating the tissue movements on the kymograph recording paper. The amplitude of contraction was recorded. The tissue was washed with Ringer's solution after every injected drug and recording was done before another drug was introduced. The time of tissue washing varied based on the behavior of the drug on the tissue. The washed tissue in the tissue bath was allowed to normalize before addition of another drug. The temperature of the tissue bath containing the tissue was always maintained at 37^0C and the bath was gassed appropriately. The same procedure was followed for both mammary gland and uterine contraction studies.

2.4 Statistical Analysis

Significant differences between means were determined using the analysis of variance (ANOVA) test, with the least significant difference (LSD) fixed at 0.05. All data analyses were done using the statistical software package SPSS for windows version 11.0 (SPSS Inc, Chicago, IL). The data are reported as mean ± SD.

3. RESULTS AND DISCUSSION

3.1 Results

Mean milk production peaked at week 2 for all groups (Fig. 1), but thereafter dropped steadily with time in all the groups. At the end of week 1, dams in the control group produced the largest quantity of milk (8.00 ± 1.0 g) while those fed with 5mg/kg of the plant extract produced the least (4.33 ± 2.89 g). The quantities of milk produced by the other treatment groups fell between these two values. The difference in the means of the control group however did not achieve statistical significance (P > 0.05) when compared to any of the treatment groups.

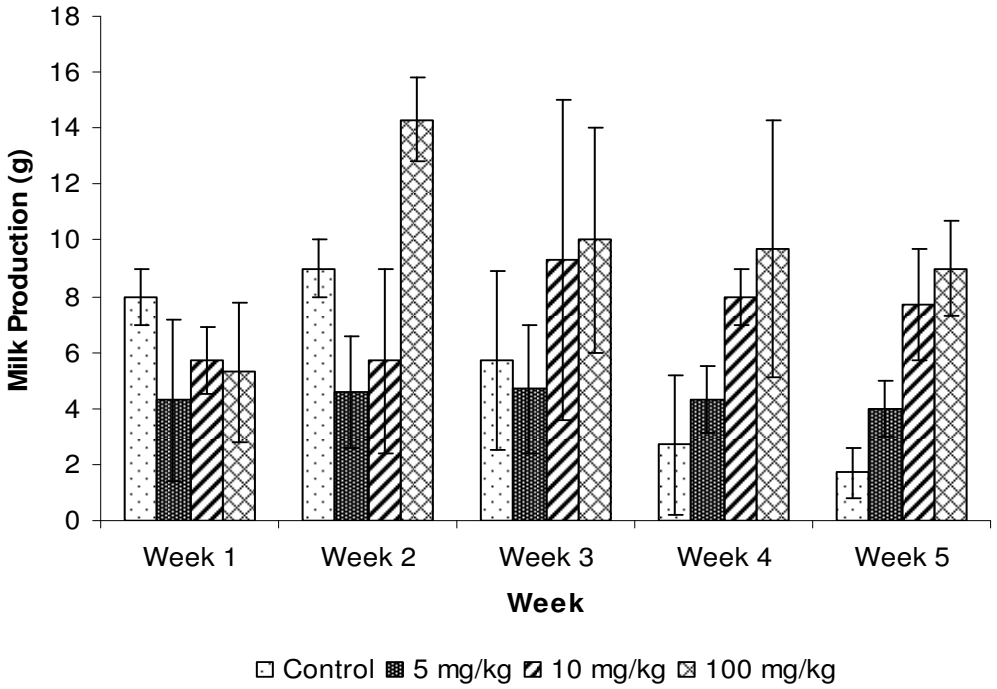

Figure 1: Mean weekly milk production (g) in dams administered *V. amygdalina* (Mean ± SD; n = 3; LSD = 0.05)

At week 2, mean milk production increased in all the groups except the control group, relative to week 1. Dams given 100 mg/kg aqueous extract produced the largest quantity of milk (14.33 ± 1.53 g) while those given 5 mg/kg of the extract produced the least quantity of milk (9.00 ± 2.00g). All the treatment groups except group II produced more milk than the control group. However the differences in the means of the treatment groups, each,

compared to the control group were not significant (P > 0.05). By week 3, the quantity of milk produced dropped, relative to week 2, but remained higher than that of week 1, except for the control group. Milk production in dams receiving the extract ranged from 10.00 ± 4.00 g in group IV to 4.67 ± 2.31 g in group II. These differences in the means of the treatment groups, each, compared to the control group were however not significant (P > 0.05).

Milk production of dams in week 4 and 5 followed the same pattern. In dams in groups II, mean milk production at week 5 fell to about the levels observed at week 1. In the control group, it fell to as low as 2.67 ± 2.52 g compared to 8.00 ± 1.00 g recorded at week 1. Milk production was not only highest (8.00 ± 1.00 g) at 100 mg/kg, but higher than that of week 1 (5.67 ± 1.15), though lower than the quantity recorded at weeks 2 and 3 (11.33 ± 5.69 respectively). The difference between the mean milk productions at 10 mg/kg were significant (p < 0.05) compared to the control group. The other treatment groups did not achieve significant differences (p > 0.05) in their means relative to the control group.

Figure 2 shows the mean weekly weight gain of litters whose mothers received *Vernonia amygdalina* extract at 5 mg/kg, 10 mg/kg and 100 mg/kg body weight. The differences in weight gain in group IV (receiving 100 mg/kg body weight) were significantly higher than those in the control group, throughout the study.

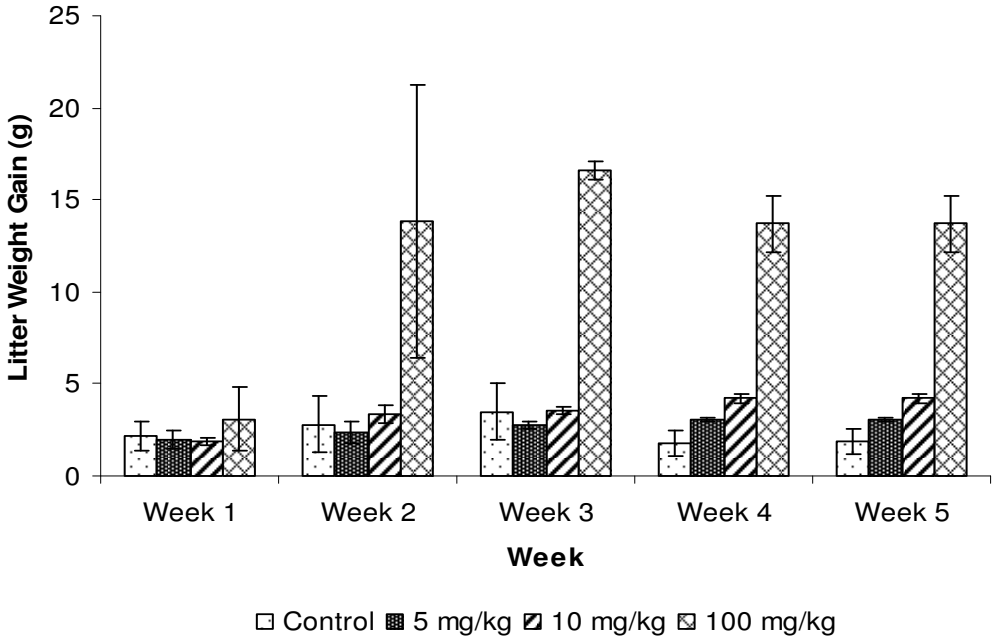

Figure 2: Mean weekly weight-gain (g) of litters administered *V. amygdalina.* (Mean ± SD; n = 3; LSD = 0.05)

Figure 3 shows that at dams in group III (10 mg/kg body weight) and IV (100 mg/kg body weight) showed statistically significant (P<0.05) weight loss.

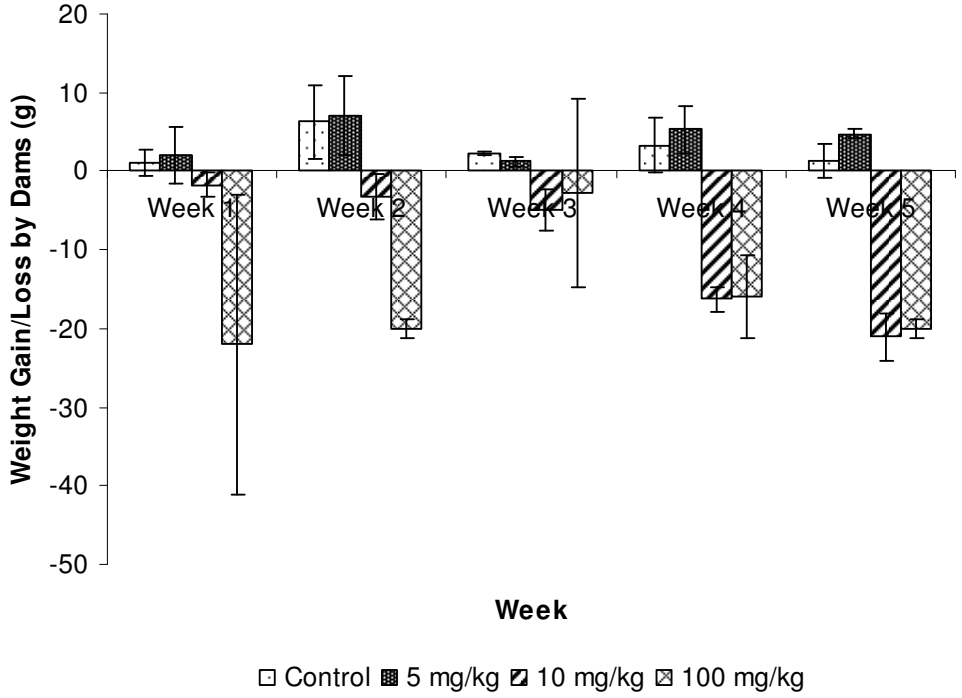

Figure 3: Mean weekly weight-gain/loss (g) of dams administered *V. amygdalina* (Mean ± SD; n = 3; LSD = 0.05)

The contraction amplitudes of aqueous extract of *Vernonia amygdalina* matched those of ergometrine only at high doses as shown in Figs 4 and 5. At 5 mg/ml *Vernonia amygdalina* gave average uterine contraction amplitude of 1.22 ± 0.03 mm as against the 2.93 ± 0.33 mm recorded for ergometrine. This gives a ratio (*Vernonia amygdalina:* ergometrine) of 1.0 : 2.4. At 10 mg/ml, the ratio became closer (1.0 : 1.3) as *Vernonia amygdalina* gave an average contraction amplitude of 3.60 ± 0.03 mm compared to the 4.68 ± 0.28 mm given by ergometrine.

The differences in the mean contraction amplitudes for both agents were significant ($p < 0.05$) at the two lower doses. At 100mg/ml the aqueous extract of *Vernonia amygdalina* matched the contraction amplitude of ergometrine (9.60 ± 0.24 mm and 9.58 ± 0.39 mm respectively) giving a ratio of 1:1. The average amplitudes of contraction for *Vernonia amygdalina* as well as for ergometrine increased significantly ($p < 0.05$) with increasing concentration of the extract.

For mammary gland contraction, the differences in the means of the contraction amplitudes, for both agents, though numerically small, remained statistically significant ($p < 0.05$) at all the test doses.

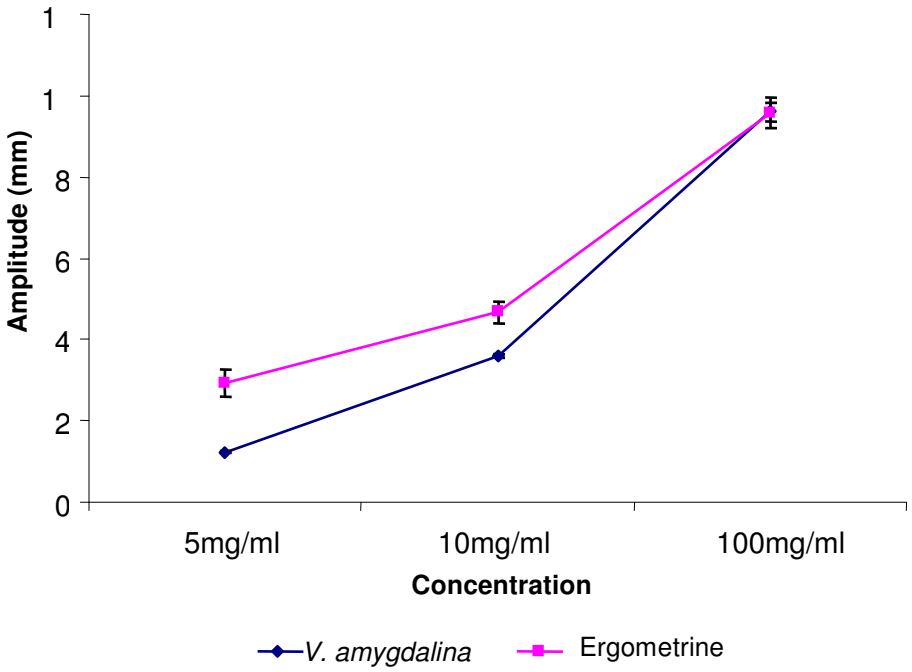

Figure 4: Uterine contraction amplitudes (mm) of dams administered *V. amygdalina* and ergometrine (Mean ± SD; n = 3; LSD = 0.05)

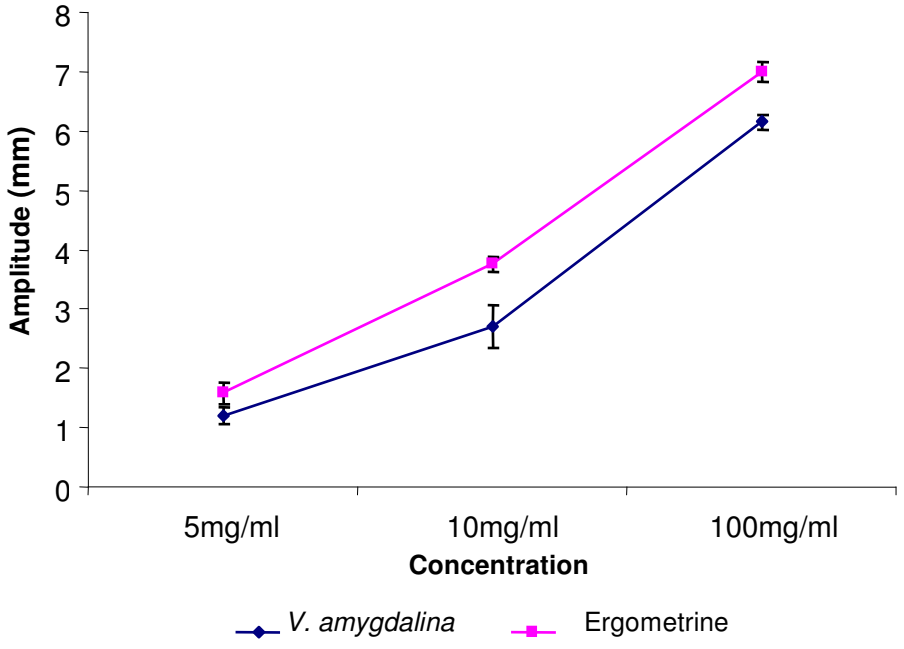

Figure 5: Mammary gland contraction amplitudes (mm) of dams administered *V. amygdalina* and ergometrine (Mean ± SD; n = 3; LSD = 0.05)

At 5mg/ml, *Vernonia amygdalina* gave average contraction amplitude of 1.22 ± 0.14 mm compared to the 1.58 ± 0.17mm recorded for ergometrine, a ratio of 1.0:1.3. At 10 mg/ml, the ratio increased slightly to 1.0:1.4, with *Vernonia amygdalina* giving an average contraction amplitude of 2.70 ± 0.36 mm at 100 mg/ml, the ratio dropped to 1.0:1.1 as *Vernonia amygdalina* almost matched the average contraction amplitude of ergometrine, (giving 6.15 ± 0.13 mm to ergometrine's 7.00 ± 0.16 mm). The noticed differences in the mean contraction amplitudes for between these agents were significant ($p < 0.05$). The average contraction amplitudes recorded for ergometrine increased significantly ($p < 0.05$) more than a hundred percent from each concentration to the next higher concentration.

3.2 Discussion

The gain in weight by litters was a reflection of the quantity of milk produced by the dams. This was observed clearly at the second and third weeks of experimentation where the bars for both litter weight-gain and milk production are almost replicas. At week 4 when milk production dropped in all groups, the litters either did not gain appreciable weight or lost some weight. This suggests that the aqueous extracts of *Vernonia amygdalina* Del not only encouraged milk production, but also induced appropriate milk let-down in a dose-dependent fashion. The capacity for *Vernonia amygdalina* Del to induce milk letdown is corroborated by the mammary gland contraction experiment. Though the aqueous extracts of *Vernonia amygdalina* Del did not match the contraction amplitudes given by the standard drug (Ergometrine), it gave good amplitudes of contraction at high doses. It is instructive that at these doses where synthetic agents such as oxytocin which has similar effects may be harmful (Litwack and Schmidt, 2002), *Vernonia amygdalina* Del has been shown to be well tolerated (Ijeh and Obidoa, 2004). The findings agree with use of *Vernonia amygdalina.* Del leaves in soups and porridges of nursing mothers in some parts of South Eastern Nigeria to encourage milk let-down. The uterine contraction studies show that *Vernonia amygdalina* Del may be useful in the induction of uterine contraction. The results suggest that at high doses, the extracts match the contractility induced by ergometrine. Oxytocics are known to induce uterine contraction, maintain and augment labour and control post partum hemorrhage (Litwack and Schmidt, 2002). This implies that the extracts may be capable of hastening parturition or causing abortion if used in preterm pregnancy. This is worrisome since the plant is used (traditionally) in the treatment of malaria and some other ailments, even in pregnancy. Our results are in discordance with the report of Bullough and Leary (1982) who reported inactivity in guinea pig uterus for the aqueous extracts of *Vernonia amygdalina* Del. Our data however agree with that of Kamatenesi-Mugisha (2004) though they studied rat uterus not guinea pig uterus.

This study appears to support the claims by trado-medical practitioners and traditional birth attendants in Nigeria that the extracts of *Vernonia amygdalina* Del are not only useful in childbirth, but also in the ejection of the placenta and the control of postpartum haemorrhage. The fact that the extracts also encourage milk production and let-down may make it very useful especially to lactating mothers. Dose and effect relationships of the extracts still require more studies and characterizations.

The data should however be interpreted with caution as *in vitro* and *ex vivo* studies do not always provide reliable guide to clinical activity. The activity of plant extracts (and generally all phytopharmaceuticals) *in vivo* reflects the interactions between multiple constituents (Gwehenberger *et al.,* 2004), that are amenable to biotransformation. The active principles in the extracts when used *in vitro* and *in vivo* may not be the same. The mechanism of action of

the extracts of *Vernonia amygdalina* Del as well as the elasticity of their receptors is still not clear. Other factors, not accounted for in this study, such as inflammatory agents (like prostaglandin) help in the initiation of contraction by potentiating the effect of oxytocin (Hayashi, 1990), thereby warranting a cautious interpretation of the data.

4. CONCLUSION

Vernonia amygdalina extracts were found to encourage milk let-down and contracted reasonably, the mammary gland and uterine muscles in dams (*ex vivo).* The results validate the use of the plant by traditional birth attendants and trado-medical practitioners.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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