World Journal of Pharmaceutical research

Volume 3, Issue 1, 29-37. Research Article ISSN 2277 – 7105

GESTATIONAL OUTCOME IN RATS: AQUEOUS *BRYOPHYLLUM PINNATUM* LEAF EXTRACTS VS. SALBUTAMOL ADMINISTRATION

***J.S. Aprioku**

Department of Pharmacology, Faculty of Basic Medical Sciences, University of Port Harcourt, Port Harcourt, Nigeria.

Article Received on
28 September 2013

Revised on 30 October 2013,
Accepted on 27 November 2013

***Correspondence for Author:**
Dr. J.S. Aprioku
Department of Pharmacology, Faculty of Basic Medical Sciences, University of Port Harcourt, Port Harcourt, Nigeria.
sydaprio@yahoo.com

ABSTRACT

Bryophyllum pinnatum Linn. is a perenial herb used extensively in folkloric medicine. The plant has been demonstrated to possess several pharmacological properties, including tocolytic activity. The present study investigates the effects of aqueous *B. pinnatum* leaf extracts (AqBP) and salbutamol, a β receptor agonist, on gestational outcome in Wistar albino rats. Pregnant rats were given AqBP (62.5 or 125 mg/kg), salbutamol (0.1 mg/kg) or vehicle (distilled water) by oral gavage from gestational day 10 until delivery. Maternal weights, length of gestation, and characteristics of delivered litters (number, weight and external appearance) were registered. Maternal weight gain was inhibited significantly ($p<0.001$) by 125 mg/kg AqBP, but AqBP (62.5 mg/kg) and salbutamol did not affect maternal weight gain. In addition, AqBP (62.5, 125 mg/kg) and salbutamol had no significant ($p>0.05$) effect on length of gestation, and also on number and external morphology of litters delivered. Furthermore, while salbutamol and the lower dose of *B. pinnatum* (62.5 mg/kg) caused no significant ($p>0.05$) effect, the higher dose of AqBP (125 mg/kg) decreased birth weight of litters. The results indicate that low dose levels of AqBP and salbutamol may not negatively affect pregnancy outcome, but the plant may adversely affect pregnancy outcome at high dose levels in the rat.

Keywords: *Bryophyllum pinnatum*, maternal weight, pregnancy, tocolytic.

INTRODUCTION

Bryophyllum pinnatum (Lam.), which belongs to the family Crasulaceae, is a succulent, perennial herb. The plant is readily cultivated from various parts, including the leaf and this may be a reason for its wide global distribution including, tropical Africa, tropical America, India, China and Australia[1]. *B. pinnatum* or *Kalanchoe pinnata* (Lam.), or *Bryophyllum calycinum* (Salisb.) as it is also called, is identified by several common names in different areas, including, air plant, life plant, love plant, miracle plant, resurrection plant, etc. In Nigeria, it is commonly called "Never die".

The leaves of *B. pinnatum* have been shown to contain pharmacologically potent phytoconstituents including, flavonoids[2], bufadienolides[3], phenols[4], organic acids[1], tannins, and alkaloids[1,5]. This may explain the extensive use of *B. pinnatum* in folkloric medicine in Africa and many other regions for the treatment of several conditions including, earache, burns, abscesses, ulcer, insect bites, diabetes, diarrhea, and lithiasis[6,7,8]. The plant is also well known for its antihypertensive, hemostatic, and wound healing properties[9,10]. A number of these folkloric claims have been established in previous studies, including its antihypertensive[10], insecticidal[3], analgesic, anti-inflammatory[11], and antimicrobial properties[2,12].

B. pinnatum has also been shown to affect uterine smooth muscles, causing inhibition of oxytocin-mediated contractions of human term myometrium strips[13,14]. The plant has been experimented in clinical studies for the prevention of preterm labor[15] and promises to be a suitable alternative to standard tocolytic agents like fenoterol and salbutamol in obstetrics. Also, in a retrospective analysis from an obstetric clinical practice, Daub[16] has reported better outcomes and less adverse effects in patients treated with *B. pinnatum* (ethanolic tincture, 33 %) compared to treatment with fenoterol. More recent studies have equally revealed that *B. pinnatum* produce similar tocolytic outcome but less adverse effects than treatment with β agonists currently used in clinic as standard therapy for prevention of pre-term delivery[17].

The present work compares the effects of aqueous *B. pinnatum* leaf extracts and salbutamol, a β receptor agonist on gestational outcome in Wistar albino rats.

MATERIALS AND METHODS

Salbutamol tablets (Glaxo Wellcome, Egypt SAE) was purchased from the Department of Pharmacy of the University of Port Harcourt, Nigeria.

Extraction of *Bryophyllum pinnatum* leaf extracts

Fresh leaves of *Bryophyllum pinnatum* were washed and air dried. The dried leaves were pulverized and extracted exhaustively in distilled water for 72 hrs by cold maceration. The leaf extracts were strained with a muslin cloth and concentrated under reduced pressure using a rotary evaporator at a temperature of 40^{o}C. The dry extracts obtained were preserved in a refrigerator at 4^{o}C until used.

Animals

Non pregnant female Wistar albino rats weighing between 170-200 g and male albino rats weighing between 200-220 g were obtained from the animal house of the University of Port Harcourt, Port Harcourt, Nigeria. The animals were housed in cages, with a 12 h light–dark cycle. They were supplied with standard laboratory chow and tap water was given *ad libitum*. The experimental protocol followed the Guide for Care and Use of Laboratory Animals[18], and was approved by the Committee for Ethics in Animal Experimentation of the University of Port Harcourt.

Experimental design

Animals were divided into different groups and allowed to mate freely. Mating was confirmed through vaginal smear examination by the presence of vaginal copulation plug (day 0 of gestation). Pregnant rats were separated and randomly distributed into 4 groups containing 8 animals per group and given different dose levels of aqueous *B. pinnatum* leaf extract (AqBP) and salbutamol daily from gestational day 10 until delivery by oral gavage. This period corresponds to the period within which preterm labor usually occurs in humans[19]. The first group was given 62.5 mg/kg AqBP. The second group received 125 mg/kg AqBP. The third group received 0.1 mg/kg salbutamol, its standard dose equivalent in the treatment of preterm labor[20]. The fourth group (control) received distilled water (0.1 ml). The body weights of pregnant animals, length of gestation, and number and birth weight of pups delivered were noted. Physical appearances of the pups were also registered by careful examination of their physical appearances (structures) for any abnormality.

Statistical analysis

Data are expressed as means ± S.E.Ms. Statistical differences between the groups were analyzed by one-way ANOVA. Comparisons between groups were made by the Newman-Keuls Multiple Comparison Test to compare maternal weights across time. Differences

yielding P-values <0.05 were considered statistically significant. Statistical analyses of data were performed using GraphPad Prism 5 software.

RESULTS

Weights of pregnant rats treated with only distilled water (controls) increased significantly ($p < 0.001$) over time during pregnancy (Figure 1). Body weights of rats given salbutamol and 62.5 mg/kg aqueous *Bryophyllum pinnatum* leaf extracts (AqBP) also increased significantly ($p < 0.0001$,$p = 0.0002$) over time, but there was no significant ($p = 0.0720$) change in the body weight of 125 mg/kg AqBP-treated pregnant rats (Figure 1). Compared to maternal body weights on day 5, the weights on day 10 were significantly, $p < 0.01$,$p < 0.05$ higher in the control and AqBP (62.5 mg/kg)-treated groups. Also, the weights on days 15 and 20 were higher, $p < 0.001$, but in the salbutamol-treated group, only the weight on day 20 was significantly, $p < 0.01$different from the weight on day 5 (Figure 1). Furthermore, maternal weights on days 15 and 20 were higher in control, $p < 0.05$,$p < 0.001$, and AqBP (62.5 mg/kg)-treated group, $p < 0.05$,$p < 0.001$; but only that of day 20 was higher in salbutamol-treated group, $p < 0.01$ when compared with the body weights on day 10. The body weights on day 20 in control, AqBP (62.5 mg/kg)- and salbutamol-treated groups were higher, $p < 0.05$ than the weights on day 15 (Figure 1).

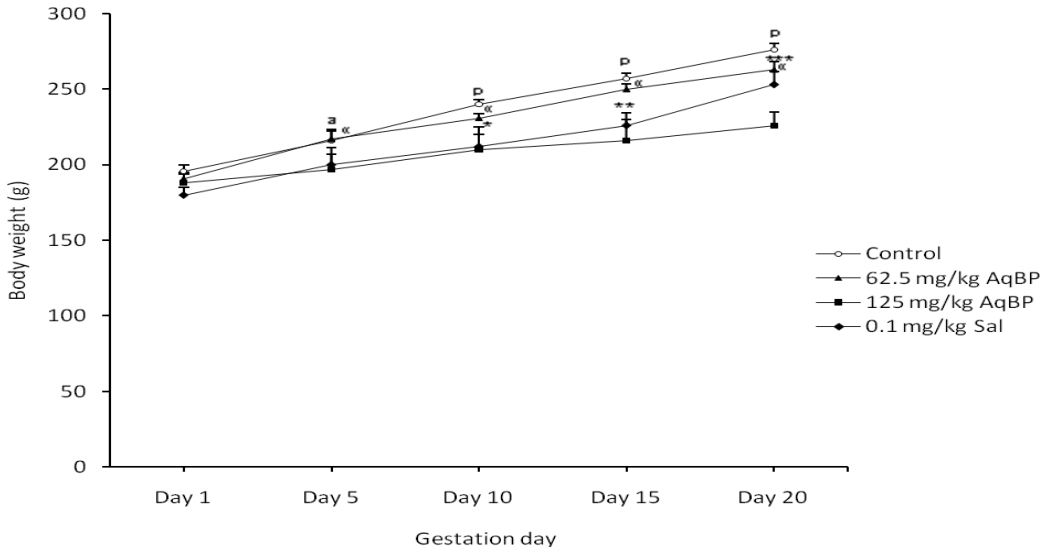

Figure 1: Effects of aqueous *Bryophyllum pinnatum* leaf extracts (AqBP) and salbutamol (Sal) on maternal body weight in Wistar albino rats

Data are expressed as mean ± S.E.M, n = 8 animals per group. Statistical differences between the groups were evaluated by one-way ANOVA, followed by Newman-Keuls Multiple Comparison Test.

In control group, [a] Significant at $p < 0.01$ (Day 1 vs Day 5), [Þ] Significant at $p < 0.001$ (Day 1 vs Days 10, 15 and 20).

In 62.5 mg/kg AqBP-treated group, [α] Significant at $p < 0.001$ (Day 1 vs Days 5, 10, 15 and 20).

In Sal-treated group: * Significant at $p < 0.05$ (Day 1 vs Day 10), ** Significant at $p < 0.01$ (Day 1 vs Day 15), *** Significant at $p < 0.001$ (Day 1 vs Day 20).

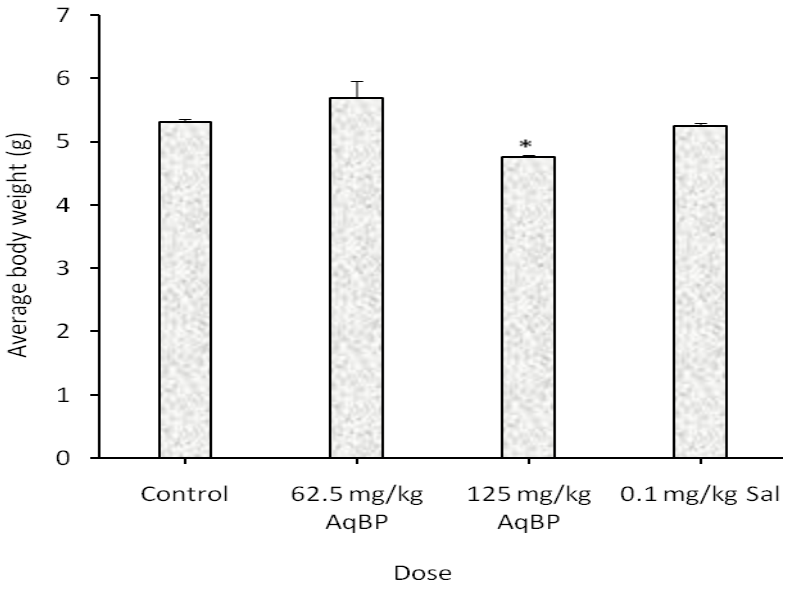

Figure 2: Effects of aqueous *Bryophyllum pinnatum* leaf extracts (AqBP) and salbutamol (Sal) on birth weight of litters in Wistar albino rats

Data are expressed as mean ± S.E.M. Data analyzed by one-way ANOVA. * Significant at $p < 0.01$.

Table 1: Effects of aqueous *Bryophyllum pinnatum* leaf extracts (AqBP) and salbutamol (Sal) on some pregnancy parameters in Wistar albino rats

Dose	Average length of gestation (Days)	Average no. of pups delivered	Physical appearance of litters
Control	20.75±0.48	9.00±0.41	Normal and active
62.5 mg/kg AqBP	24.00±1.47	8.50±0.50	“
125 mg/kg AqBP	21.25±0.75	8.25±0.63	“
0.1 mg/kg Sal	24.75±1.44	7.50±0.29	“

Data are expressed as mean ± S.E.M.

Length of gestation in AqBP (62.5, 125 mg/kg)- and salbutamol-treated rats were not significantly ($p > 0.05$) different from control rats (Table 1). There were also no significant differences in the number of litters delivered by AqBP (62.5, 125 mg/kg)- and salbutamol-treated animals, compared to control animals (Table 1). Furthermore, while pup birth weights of salbutamol- and AqBP (62.5 mg/kg)-treated mothers were not significantly different, pup weights of AqBP (125 mg/kg)-treated mothers were significantly ($p < 0.01$) lower, compared to controls (Figure 2). Pups of AqBP (62.5, 125 mg/kg)- and salbutamol-treated mothers were active and there were no morphological abnormalities, compared to controls.

DISCUSSION

Salbutamol is a betametic agent and acts as uterine muscle relaxant (tocolytic agent), by stimulating β_2 adrenergic receptors in uterine smooth muscle. Salbutamol is used to prevent preterm labor in obstetrics, which generally occurs between 24 and 37 weeks of gestation[19]. *B. pinnatum* is a widely studied herbal plant, used in folkloric medicine for the treatment of different conditions[6,8,9]. The extracts of *B. pinnatum* leaf has been demonstrated to have tocolytic property, through inhibition of oxytocic action in gravid uterus[13,14], and it has been tested in humans for this purpose[16]. The plant has also been reported to have lesser adverse effects than β receptor agonists when used for this purpose[16,17]. The present study reports the effects of aqueous *B. pinnatum* leaf extracts and salbutamol on gestational outcome in the rat.

In previous experimental studies on *B. pinnatum*, most pharmacological activities, including analgesic and anti-inflammatory were observed at dose levels of 200 mg/kg and above[11,12]. However, Plangger et al.[17] have demonstrated tocolytic activity of *B. pinnatum* in humans at a dose of 30 mg, given intravenously as an infusion. In the present study, oral dose levels of 62.5 and 125 mg/kg weight per day were used since uterine tissues may be more sensitive to the plant than other issues.

In this study, the higher dose of *B. pinnatum* leaf extracts (125 mg/kg) prevented maternal weight gain, while the lower dose of *B. pinnatum* leaf extracts (62.5 mg/kg) and salbutamol did not affect normal increase in maternal weight during pregnancy. Weight gain in pregnancy is essential for normal fetal development and well being of mother[21]. Furthermore, inadequate maternal weight gain during pregnancy has been linked to low birth weights and this is associated with high rates of neonatal diseases and survival[22]. This suggests that administration of high dose levels of *B. pinnatum* leaf during pregnancy may affect normal fetal development and growth.

It was also observed that *B. pinnatum* leaf extracts (62.5, 125 mg/kg) and salbutamol had no effect on length of gestation, number of litters delivered, and external morphology of litters. Additionally, while *B. pinnatum* (62.5 mg/kg) and salbutamol had no significant ($p > 0.05$) effect, the higher dose of *B. pinnatum* leaf extracts (125 mg/kg) reduced birth weight of litters. This correlates with the maternal weight gain inhibition by *B. pinnatum* (125 mg/kg) observed in this study. Low birth weight has been shown to positively correlate with negative consequences on the health of the offspring, with development of respiratory, metabolic and cardiac diseases in later stages of life[23]. This suggests that administration of high dose levels of *B. pinnatum* leaf extracts in pregnancy may negatively affect outcome of pregnancy in the rat.

CONCLUSION

From the results of this study, it can be concluded that lower dose levels of *B. pinnatum* may have no adverse effects on pregnancy outcome, similar to the effect of salbutamol, but high dose levels of the plant adversely affects pregnancy outcome in the rat.

ACKNOWLEDGEMENTS

Author is grateful to the laboratory staff of the Department of Pharmacology, University of Port Harcourt, Port Harcourt, Nigeria for their technical assistance.

REFERENCES

1. Afzal M, Kazmi I, Khan R, Singh R, Chauhan M, Bisht T, Anwar F. Bryophyllum pinnatum: A review. Int J Res Biol Sci, 2012; 2(4): 143-9.
2. Okwu DE, Nnamdi FU. Two novel flavonoids from Bryophyllum pinnatum and their antimicrobial activity. J Chem Pharm Res, 2011; 3(2): 1-10.
3. Kamboj A, Saluja AK. Bryophyllum pinnatum (Lam.) Kurz.: Phytochemical and pharmacological profile : A review. Pharmacogn Rev, 2009; 3(6): 364-74.
4. Asiedu-Gyekye IJ, Antwi DA, Bugyei KA, Awortwe C. Comparative study of two kalanchoe species: total flavonoid, phenolic contents and antioxidant properties. Afri J Pure Appl Chem, 2012; 6(5): 65-73.
5. Adenike K, Eretan OB. Purification and partial characterization of a lectin from the fresh leaves of *Kalanchoe crenata* (Andr.) Haw J Biochem Mol Biol, 2004; 37(2): 229-33.
6. Gill LS. Ethnomedical uses of plants in Nigeria. Nigeria; University of Benin Press: 1992.

7. Chopra RN, Nayar SI, Chopra II. Glossary of Indian medicinal plants. New Dehli, India; NISCIR (CSIR): 2002.
8. Lans CA. Ethnomedicines used in Trinidad and TobagoTrinidad and Tobago for urinary problems and diabetes mellitus. J Ethnobiol Ethnomed, 2006; 2: 45.
9. Oliver-Bever B. Medicinal plants in tropical Africa. J Ethnopharmacol, 1983; 9: 1-83.
10. Ghasi S, Egwuib C, Achukwu PU, Onyeanusi JC. Assessment of the medical benefit in the folkloric use of *Bryophyllum pinnatum* leaf among the Igbos of Nigeria for the treatment of hypertension. Afr J Pharm Pharmacol, 2011; 5(1): 83-92.
11. Ojewole JAO. Antinociceptive, anti-inflammatory and antidiabetic effects of bryophyllum pinnatum (Crassulaceae) leaf aqueous extract. J Ethnopharmacol, 2005; 99(1): 13-9.
12. Quazi MA, Nazim S, Afsar S, Siraj S, Patel MS. Evaluation of antimicrobial activity of roots of Kalanchoe pinnata. Int J Pharmacol Bio Sci, 2011; 5(1): 93-6.
13. Gwehenberger B, Rist L, Huch R, von Mandac U. Effect of Bryophyllum pinnatum versus fenoterol on uterine contractility. Eur J Obstet Gynecol Reprod Biol, 2004; 113: 164-71.
14. Simoes-Wust AP, Graos M, Duarte CB, Brenneisen R, Hamburger M, Mennet M, Ramos MH, Schnelle M, Wächter R, Worel AM, von Mandach U. Juice of Bryophyllum pinnatum (Lam.) inhibits oxytocin-induced increase of the intracellular calcium concentration in human myometrial cells. Phytomedicine, 2010; 17(12): 980-6.
15. Hassauer W, Schreiber K, Von der Decken D. Ein neuerWegin der tokolytis-chen Therapie. Erfahrungsheikunde, 1985; 34: 683-7.
16. Daub E. Vorzeitige Wehentatigkeit. Ihre Behandlung mit pflanzlichen Substanzen, eine klinische Studie. Stuttgart; Urachhaus: 1989.
17. Plangger N, Rist L, Zimmermann R, von Mandach U. Intravenous tocolysis with Bryophyllum pinnatum is better tolerated than beta-agonist application. Eur J Obstet Gynecol Reprod Biol, 2006; 124(2): 168-72.
18. CCAC- Canadian Council On Animal Care. The Care and Use of Farm Animals in Research, Teaching and Testing. Ottawa: 2009.
19. Goldenberg RL, Culhane JF, Lams JD, Romero R. Epidemiology and causes of preterm birth. The Lancet, 2008; 371(9606): 75–84.
20. Junejo N, Mumtaz F, Unar BA. Comparasion of salbutomol and nefidepine as a tocolytic agent in the treatment of preterm labour. J Liaquat Uni Med Health Sci, 2008; 7: 115-9.

21. Derbyshire E. Low maternal weight: effects on maternal and infant health during pregnancy. Nurs Stand, 2007; 22(3): 43-6.
22. Kramer MS. Determinants of low birth weight: methodological assessment and meta-analysis. Bull World Health Organ, 1987; 65: 663-737.
23. Barker DJ, Gluckman PD, Godfrey KM, Harding JE, Owens JA, Robinson JS. Fetal nutrition and cardiovascular disease in adult life. Lancet, 1993; 341: 938-41.