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PHYTOCHEMICAL CONSTITUENTS AND UTEROTONIC EFFECTS OF AQUEOUS EXTRACT OF *CALOTROPIS PROCERA* LEAVES ON EXCISED UTERINE SEGMENTS OF ALBINO RATS

Shamaki, B. U[1*], William. A[1]., Abdullahi, A[1] and Sadiq, A. A[1].

[1]Department of Veterinary Physiology, Pharmacology and Biochemistry. Faculty of Veterinary Medicine, PMB 1069. University of Maiduguri. Nigeria.

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***Correspondence for Author**

Shamaki, B. U

Department of Veterinary Physiology, Pharmacology and Biochemistry. Faculty of Veterinary Medicine, PMB 1069. University of Maiduguri. Nigeria.

ABSTRACT

The phytochemical screening and uterotonic effects of aqueous extract obtained from *Calotropis precera* leaves were investigated in attempt to evaluate its medicinal potentials. The phytochemical screening revealed the presence of carbohydrates, tannins, cardiac glycosides, flavonoids, terpenoids and saponin glycosides. Albino rats uterine segments were exposed to graded bath concentration of the aqueous extract of *Calotropis procera* leaves and the responses were recorded kymographycally at 37^{o}C. The results revealed increase in amplitude of contraction with increase in concentration of the stock solution of the extract although the increase is not significant ($p>0.05$). Similarly, the oxytocin response shows gross increase with increasing concentration of the stock solution but also not significant ($p>0.05$). Furthermore, there was no significant variation ($p>0.05$) between and among the mean responses of oxytocin and the aqueous extract of *Calotropis procera* leaves. In conclusion, this study suggests that aqueous extract of *Calotropis procera* have abortifatient effect by stimulating a spontaneous contraction on the myometrium.

Keywords: Uterotonic, Uterine segments, *Calotropis procera*, aqueous extract, Albino rats.

INTRODUCTION

Medical abortion has emerged as a valuable alternative to surgical abortion and will contribute to safe reproduction control worldwide (Reynolds, 1996; Gan *et al*., 2008). Although synthetic abortifacients of known biochemical mechanisms are effective and

popular, but the risk associated with these drugs have triggered the need to develop new molecule from medicinal plants. Hence, there is a need for elucidating the physiological and biochemical mechanisms of suitable natural products with known abortifacient property from indigenous medicinal plants that could be used as therapeutic alternatives for women in developing world.

Calotropis procera (Asclepiadaceae) is a perennial shrubby treelet with thick cottony tomentose leaves when young and frequently glabrescent when fully developed (Huber, 1985). The leaves are opposite, sessible, oblong-obovate, short pointed to blunt at the apex and are about 7 to 18 cm long and 5 to 13 cm broad, slightly leathery and have a fine coat of soft hair. The flowers are white to pink or spotted purple. The fruit are inflated, 1-12 cm long, grey-green color and release flat, brown seeds with a tuft of white hair at one end. The roots are simple, whitish-grey in color with wrinkles, curved woody appearance and exhibit marks of sap exudation on the surface. The root is bitter in taste and has no specific odour. The aerial part of the plant contains milky sap called latex present in the lactiferous channels (Rahman and Wilcock, 1991). The generic name is taken from kalos (beautiful) and Tropis (a keel), alluding to the good look of the keel of flower. It is distributed widely in the tropics, especially in the waste places (Hussein *et al*., 1994). It is a common plant in Nigeria called "bom bom" but it is more abundant in the northern part of the country (Sofowora, 1994; Mbako *et al., 2009*). *Calotropis procera* is known by varios names like Dead Sea apple, Sodom apple, swallow wort and Milkweed (Gupta *et al*., 2012).

Phytochemically, the plant has been investigated for cardenolide from the latex and leaves of plants, triterpenoids, anthrocyanins from flowers and hydrocarbons. The leaves and latex of *Calotropis procera* were found to have cardiac glycosides which include calotrogenin, calotropin, uscharin, calotoxin, calactin (Al-Robal *et al*., 1993; Mueen *et al*., 2005) Alkaloids, flavonoids sterols have also been found to be present in the entire plant (Edman, 1983; Hussein *et al*., 1994).

Most elucidated abortifacient mechanisms involve contractility of the uterine smooth muscle. In smooth muscle, β-adrenoreceptors stimulation decreases contractility, while stimulation of α_1-adrenoceptors increases smooth muscle contractility. (Michael, 2007).

In this study, the effect of aqueous leave extract of *Calotropis procera* on the contractility of uterine segment was determined.

MATERIALS AND METHODS

Sample collection

Fresh leaves of *Calotropis procera* were collected on 6th March 2014 early in the morning in the university of Maiduguri campus, Maiduguri, Borno state. The plant was identified and authenticated at the Herbarium of Biological Sciences, University of Maiduguri. The leaves were brought to the laboratory for air drying under room temperature [32 ± 2^0c] to reduce the moisture content before processing.

Plant extraction

Exactly 200 g of dried sample materials were pulverized using wooden mortar and pestle. The sample was transferred into 2 liter round bottom flask and sufficient amount of distilled water was added until it covered the sample. The mixture was refluxed for about 2 hours. It was removed and the solution was decanted. This was repeated twice using a new solvent (Distilled water). After the sample was extracted, the solution was filtered and concentrated in hot air oven at 40-50^{0}C. After evaporating, a dark green substance was obtained. It weighted 116 g with pH of 4.2 and was transferred into an air tight container for further analysis.

Preliminary phytochemical screening of the extract

Preliminary qualitative phytochemical analyses for constituents such as carbohydrates, monosaccharide, reducing sugars, free reducing sugars, combined reducing sugars, glycosides, alkaloids, combined anthraquinone, tanins, phlobatannins, were done as described by Trease and Evans (2002), while test for ketoses, pentoses and soluble starch according to method of Vishnoi (1979), cardiac glycosides, steroids and terpenoids by method of Silva *et al* (1998), saponin glycosides (Sofowora, 1993) and flavonoids as described by Markham (1982)

Preparation of stock solution

The stock solution was prepared by dissolving 1g of the extract in 10ml of distilled water. The stock solution was used within a few hours of its preparation.

Experimental protocol

The rats were humanely sacrificed and exsanguinated post parturition. The abdominal cavity was cut open; the uterus was located, excised and placed in a beaker containing aerated Dejalon's solution. Subsequently, the uterus was dissected and freed from surrounding tissue

and mounted in 75 ml organ bath (Scientific and research instrument Ltd, England) filled with Dejalon's solution and left to equilibrate at the bath temperature of 37^{o}C.

The solution was constantly aerated with an aerator. The set-up was linked to a kymograph (Harvard Instrument, USA) via a frontal writing lever with resting tension if 2 g. Isometric contractions of the segments were first recorded with the kymograph speed set at 0.25 mm/sec. Subsequently the segments were exposed to range of stock solutions from 0.2 ml to 1 ml, the responses recorded by kymograph and concentration-response curve were constructed. Each dose level was allowed to act for 10-15 seconds before being washed off with Dejalon's solution by drainage and refilled with the solution.

Statistical analysis

Student's 't' test was used to compare the amplitude of mean ±SEM of the extract treated and 10 IU/ml Oxytocin treated uterine segments at 95 % confidence interval. All calculations and statistical analyses were done using the computer software GraphPad InStat Version 3.0

RESULTS

The results for the phytochemical screening tests are presented in table 1. Positive test were recorded in general; carbonhydrates, tannins, cardiac glycosides, flavonoids, terpenoids, saponin glycosides were found positive.

Mean uterine responses (contraction amplitude) to the exposure to oxytocin and aqueous extract of *Calotropis procera* leaves at graded bath concentrations are provided in Table 2. The values for the amplitude of contractions increase although not significant ($p>0.05$) with increasing concentration of the stock solution. Similarly, the oxytocin responses showed gross increase with increasing concentration of the stock solution. However, this increase was also not significant ($p>0.05$).

There was no significant variation ($p>0.05$) between and among the mean responses of oxytocin and the aqueous extract of *Calotropis procera* leaves (figure 1).

Table 1: Qualitative phytochemical constituents

Phytochemical	Test	Remark
Carbonhydrates	General molisch test	+
	Test for free reducing sugar	-
	Test for combined reducing sugar	-
	Test for ketoses	-
	Test for pentose	-
Tannins	Ferric chloride test	+
	Lead acetate test	+
Phlobatannins	Test for phlobatannins	-
Glycosides	Test for free anthraquinones	-
	Test for combined anthraquinone	-
Cardiac glycosides	Salkwosky test	+
	Leibeman-Burchard test	+
Flavonoids	Shinoda's test	+
	Ferric chloride test	+
	Lead acetate test	+
	Sodium Hydroxide	-
Terpenoids	Test for terpenoids	+
Saponin glycosides	Frothing test	+
Alkaloids	Dragendroff's reagent	-
	Mayer's reagent	-

Key: - = absent, + = present.

Table 2: Mean (±SEM) amplitude of contraction of uterine segments exposed to graded bath concentrations of aqueous extract of *Calotropis procera* leaves.

Final bath concentration of the extract (mg/ml)	Amplitude of contraction (mm) Extract treated segments	Amplitude of contraction(mm) Oxytocin treated segments
0.26	12.0±2.3	16.7±1.2
0.53	15.9±4.1	17.3±0.8
0.80	17.4±4.3	18.7±1.5
1.07	19.6±4.4	20.0±1.2
1.30	**21.9±4.2**	**20.7±2.3**

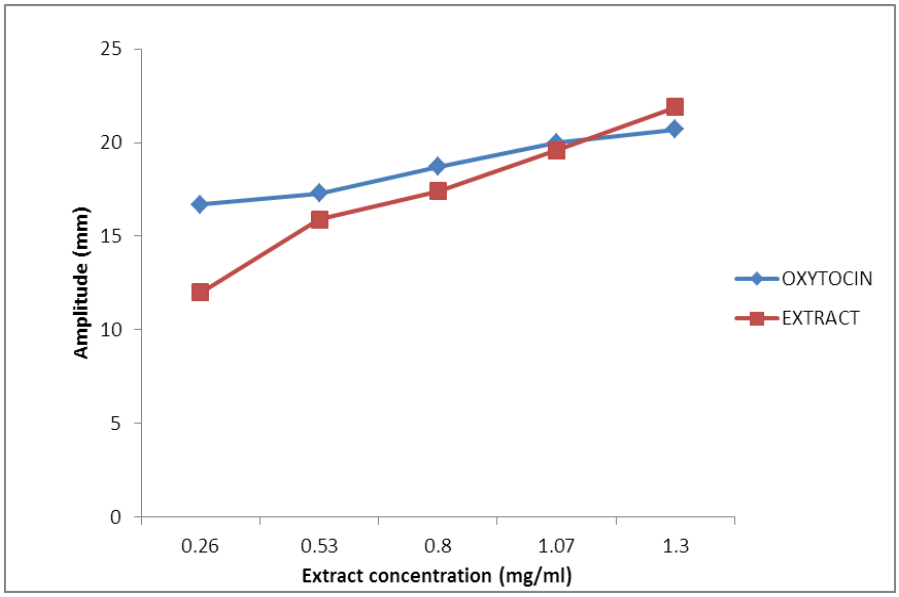

Figure 1: Cumulative dose-amplitude curve of aqueous extract of *Calotropis procera* leaves and oxytocin

DISCUSSION

The present study demonstrates the uterotonic effects of aqueous extract of *Calotropis procera* leaves on uterine segments. The observed uterotonic response by the extract in this study was characterized by an increase in the amplitude of uterine contractions indicating the abortifacient effect of the Extract. However the contractile effect was not significantly different with that of oxytocin. This means that the uterotonic effect of the extract produced an equal maximal effect on the uterine muscle segments with that of oxytocin. This is similar to findings reported by James *et al.,* (2011). It has also been described by Saha *et al*, (1961); Mahli and Trivedi, (1972); Jain *et al*, (1996); El-Badwi and Bakheit, (2010) to have abortifatient effect by stimulating a spontaneous contraction on the myometrium. The possible biochemical mechanism of action is by binding to β_2-adrenergic receptors thereby causing a decrease in the level of cAMP. cAMP reduction reduces the activation of Protein Kinase A (PKA) thereby preventing PKA from inactivating Myosin Light Chain Kinase thereby inducing myometrial contractions of the uterus (James *et al*., 2011).

The presence of phytochemical such as carbonhydrates, tannins, cardiac glycosides, flavonoids, terpenoids, saponin glycosides which corresponds to finding by Edman, (1983), Gupta *et al.,* (2003) and Jato *et al.,* (2009). The presence of these components in leaves is indication that it may perhaps have some medicinal or toxic potential. This is probably due to the fact that each of the components identified has one therapeutic usage or another.

Therapeutic uses of cardiac glycosides such as calotropin and cardenolide have been adapted for the treatment of congestive heart failure and cardiac arrhythmia. Their utility results from an increased cardiac output by increasing the force of contraction. By increasing intracellular calcium, cardiac glycosides increase calcium-induced calcium release and thus contraction (Singh and Rastogi, 1970). This is in line with prostaglandins and oxytocin, which stimulate uterine contractions by binding to specific receptors on the myometrial-cell surface (Izumi *et al*., 1994). This action results in increased calcium production by the endoplasmic reticulum and consequently, increase in uterine contraction (Izumi *et al*., 1994). Other previous studies also demonstrate that calotropin can be used as a potent abortifatient or interceptive agent for unwanted pregnancies (Gupta *et al.,* 1990).

Tannins have astringent property which makes the intestinal mucosa more resistant and reduces the secretion by contracting the gastrointestinal tract, this agrees with the report of Kumar *et al.,* (2001) on the antidiarroeal effect of *Calotropis procera* extract. The anti-inflammatory effect of tannins help in the control of all indication of gastritis, esophagitis, enteritis and irritating bowel (Hemingway and Karchesy, 1989) which also coincides with the report of anti-inflammatory effect of *Calotropis procera* by Majumder *et al.,* (1997) and Kumar *et al.,* (1994) respectively.

Flavonoids are known to relax pre-contracted intestinal smooth muscle and to delay intestinal transit. Flavonoids posses antibacterial, spasmolytic properties, this make *Calotropis procera* extract to have antibacterial and spasmolytic activity as reported by Sharanu *et al.,* (2007).

The presence of terpenoids has antihistaminic properties and hepatoprotective properties (Rastogi and Mehrota (1993) and responsible for the anti-allergic property of the extract.

CONCLUSION AND RECOMMENDATION

In conclusion, this study shows the uterotonic effect of *Calotropis procera* leaves with a tendency of causing abortion in animals. Therefore, it can be a valuable alternative to surgical abortion and will contribute to safe reproductive control worldwide. However, it should be avoided in animals feed and prevent animals from grazing in areas inhabited with *Calotropis procera* because of the possible abortifatient effect of the leaves

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