

Aqueous Root Extract of the Anti-Malarial Herbal *Cryptolepis sanguinolenta* (Lindl) Schltr Inhibits Ovulation in Rabbits

Charles Ansah and Kwesi Boadu Mensah

Department of Pharmacology, College of Health Sciences, Kwame Nkrumah University of Science and Technology, Kumasi, Ghana

Abstract: The objective of the present study was to assess the effect of the aqueous root extract of *Cryptolepis sanguinolenta* (cryptolepis), a popular West Africa antimalarial agent on ovulation in rabbits. We demonstrated previously that the aqueous root extract of the plant inhibits reproduction and foetal development in mice. The possible mechanism involved in this reproductive effect and whether the effect is species dependent has yet to be elucidated. Using the rabbit, the present study examined the effect of cryptolepis on pre and post ovulatory events to explore further the mechanism of the reproductive toxicity of cryptolepis. Cryptolepis (62.5-500 mg/kg; p.o) administered five hours before ovulation inhibited ovulation in rabbits as ovarian histology showed dilated but unruptured ovum. The effects of cryptolepis were similar to diclofenac, an NSAID used as a reference drug. Post ovulatory treatment with cryptolepis (62.5-500 mg/kg; p.o) had little effect on ovarian histology but decreased the implantation index. This study shows that the anti-conceptive effects of cryptolepis involve inhibition of ovulation and early post ovulatory events in the rabbit.

Keywords: Cryptolepis, ovulation, rabbits, reproductive toxicity

INTRODUCTION

According to WHO malaria is still endemic in Africa and majority of the people depend heavily on herbal remedies (WHO,2011). *Cryptolepis sanguinolenta* is a popular West and Central Africa antimalarial (Michel *et al.*, 2008).Recent clinical studies on the antimalarial action of the aqueous root extract (cryptolepis) in Ghana showed very promising results (Bugyei *et al.*, 2010). Apart from the established antimalarial activity, aqueous extract of the dried roots of the plant (cryptolepis) is used to treat various forms of fever, urinary and upper respiratory tract infections, rheumatism and venereal diseases (Boakye-Yiadom, 1979; Michel *et al.*, 2008). Cryptolepine is the major alkaloid in the aqueous extract (Dwuma-Badu *et al.*, 1978) and is now known to be responsible for most of the biological effects.

We reported previously (Ansah *et al.*, 2010) that aqueous root extract of *Cryptolepis sanguinolenta* inhibits reproduction and foetal development in mice. However, the mechanism involved in this action and whether it is a species dependent effect is not fully known. To further explore the possible mechanisms, we studied the effects of cryptolepis on ovulation in rabbits.

Ovulation is an important step in reproduction in the female preceding other processes such as

fertilization, implantation, gravidity and parturition. Ovulation has also been shown to be very analogous to inflammation with both processes characterized by hyperaemia, reddening, leucocytes infiltration and induction of COX 2 (Espey, 1980; Espey, 1994a; Espey and Lipner, 1994b). Consequently, substances with potent anti-inflammatory activity inhibit ovulation.

The rabbit is genetically different from the mouse and used extensively in reproductive toxicity studies (Foote and Carney, 2000).Rabbits as *induced ovulators* have ovulation occurring 10 to 12 hours after mating making it possible to study separately the effect of a chemical before or after ovulation. In this study cryptolepis is investigated for its effects on ovulation in rabbits.

MATERIALS AND METHODS

All experiments were conducted between October and December 2010 at the Department of Pharmacology, College of Health Sciences, Kwame Nkrumah University of Science and Technology, Kumasi, Ghana.

Plant material: Authenticated dried *Cryptolepis sanguinolenta* roots were obtained from the Centre for Scientific Research into Plant Medicine,

Mampong-Akwapim, Ghana where it is routinely used as an antimalarial at the clinic for patients. To simulate the traditional method of preparation, dried cryptolepis roots (1 kg) was milled and extracted by boiling with 10 litres of distilled water for 30 minutes. The solution was filtered and the filtrate after cooling was freeze-dried to obtain a yellowish-brown powder referred to subsequently as cryptolepis. The percentage yield was 9.3% w/w. Routinely, cryptolepis was freshly prepared in water and administered by gavage to the experimental animals.

Chemicals: Diclofenac injection 3 mg/ml was purchased from Denkharm, Germany

Animals: Virgin female New Zealand white rabbits(8 months;2.1-2.5 kg) were obtained from (St. Louis,Oduom, Kumasi) and kept in the animal house of the Department of Pharmacology for two weeks for acclimatization. Animals were fed on grass and administered water *ad libitum*. The animals were humanely handled throughout the experiment in accordance with internationally accepted principles for laboratory animal use and care (EEC Directive of 1986: 86/609 EEC). Additionally, all animal experiments were approved by the departmental ethics committee.

On the day of the experiment, the female rabbits were mated and the time of mating recorded.Vaginal smears were collected and examined under a microscope to confirm the presence of spermatozoa before animals were selected for the study.

Preovulatory experiment in female rabbits: In the preovulatory study, 25 mated rabbits were randomly assigned to one of five groups (n = 5). Group 1 served as vehicle control for both pre and post ovulatory experiment receiving only distilled water. Group II rabbits received (3.0 mg/kg; *i.m*) of diclofenac 5 h after mating. Groups III,IV, V rabbits received (62.5,100 or 500 mg/kg; *p.o*) of cryptolepis respectively 5 h after mating.

Post ovulatory experiment in female rabbits: In the post ovulatory study, 20 mated rabbits were randomly assigned to one of 4 groups (n = 5).Group I from

the preovulatory experiment receiving distilled water only served as control for this experiment. Group II received (3 mg/kg; *i.m*) of diclofenac 24 h after mating and this was repeated 48 hours after mating. Group III,IV,V rabbits received 62.5, 100 or 500 mg/kg; *p.o*) of cryptolepis, respectively 24 h after mating and this was repeated 48 h after mating.

All rabbits were then kept and fed normally until gestation day 10. On gestation day 10, they were euthanized. The total number of implants, number of resorption sites and the number of fresh corpora lutea were then counted.

RESULTS AND DISCUSSION

Reproductive toxicity studies require that an effect seen in one kind of animal be demonstrated in another animal of a different species. This increases the likelihood that the observed effect can occur in humans. The rabbit, aside being genetically different from the mouse, possesses some advantages over other animals used in reproductive toxicity studies. The inhibitory effects observed previously with cryptolepis on reproduction in mice (Ansah *et al.*, 2010) prompted us to investigate the effect of the extract in rabbits. The results of the preovulatory study showed that *Cryptolepis sanguinolenta* inhibits ovulation in rabbits. Treatment of rabbits with cryptolepis (62.5 to 500 mg/kg; *p.o*) or diclofenac (3.0 mg/kg; *i.m*) 5 h after mating led to an inhibition in ovulation. The decreased number of corpora lutea indicated reduced numbers of ova released (Table 1). The implantation indices for each dose of treatment were less than the control (Table 1). There were also preimplantation losses for both cryptolepis and diclofenac. Histology of the ovary showed there were dilated unraptured Graafian follicles in the ovary ten days after mating (Fig. 1). The effects observed for cryptolepis were similar to that of diclofenac.

Ovulation occurs following a drop in the mid cycle surge in luteinizing hormone (LH). Gonadotropins particularly LH mediates COX-2 induction which precedes ovulation (Sirois *et al.*, 1992; Sirois *et al.*, 2004). Consequently, preovulatory follicle exhibits symptoms of redness, swelling and pain as well

Table 1: Effects of cryptolepis on preovulatory events in female rabbits

Parameter	Control	Diclofenac	Cryptolepis		
		(3.3 mg/kg)	(62.5mg/kg)	(100mg/kg)	(500mg/kg)
Initial weight	2.34 ± 0.15	2.26 ± 0.11	2.16 ± 0.26	2.36 ± 0.11	2.18 ± 0.16
Final weight	2.49 ± 0.18	2.32 ± 0.09	2.25 ± 0.28	2.420 ± 0.10	2.32 ± 0.18
Weight gain	0.15 ± 0.03	0.59 ± 0.81	0.09 ± 0.03	0.33 ± 0.11	0.22 ± 0.11
No of corpora lutea	8.2 ± 1.30	1.4 ± 0.55***	4.2 ± 1.79***†	2.0 ± 1.58***	2.6 ± 1.95***
No of implants	6.6 ± 1.14	1.0 ± 0.71***	3.2 ± 1.10***	1.3 ± 0.96***	1.9 ± 1.14***
Implantation index (%)	80	71.4	76.2	65.0	73.0
Preimplantation loss (%)	19.5	28.5	23.8	35.0	27.0

Implantation Index = number of implants x 100 Preimplantation loss = number of corpora lutea – number of implants x 100 number of corpora lutea number of corpora lutea ; Results presented as mean ± SEM. Statistical analysis is by one way ANOVA using Neuman-Keuls post hoc test. ***means p<0.001. † means p<0.05 compared with diclofenac.

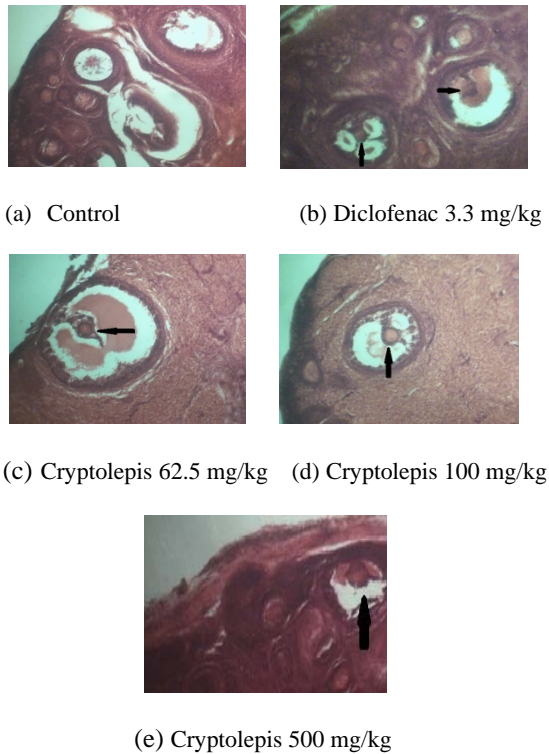


Fig. 1: Effects of preovulatory cryptolepis treatment on ovarian histology. Arrows show dilated but unruptured Graafian follicle after cryptolepis or diclofenac treatment five hours before ovulation. Control animal shows corpora lutea

as vasoactive substances, eicosanoids, interleukins, tumour necrosis factor and chemotactic substances (Chun *et al.*, 1993; Espey, 1994a), which mediate the inflammatory episode. Inhibition in ovulation may be due to endocrine and non-endocrine factors. Most endocrine ovulatory inhibitors such as hormonal contraceptives exert a negative feedback inhibition on the hypothalamus-hypophysis-gonadal axis thereby deregulating gonadotropin release.

However due to the role of COX-2 in ovulation, substances with potent anti-inflammatory activity such as non-steroidal anti-inflammatory agents (NSAIDs) inhibit ovulation. COX-2 selective inhibitors induce anovulatory condition in mammals where there are

clinical signs of ovulation but there is no ovum release (Salhab *et al.*, 2001). This has also been observed in young women with rheumatoid arthritis on NSAIDs undergoing fertility treatments (Zanagnolo *et al.*, 1996; Skomsvoll *et al.*, 2005). Furthermore, this has also been confirmed in experiments using COX-2 knockout mice. Mice lacking the COX-2 gene have multiple reproductive, ovulatory and fertilization failure (Lim *et al.*, 1997).

Cryptolepis and cryptolepine, the main alkaloid in the aqueous extract, possess potent anti-inflammatory activity mediated by COX-2 inhibition (Bangbose and Noamesi, 1981; Olajide *et al.*, 2007a, b; Olajide *et al.*, 2009; Olajide *et al.*, 2010). In addition cryptolepine directly blocks the activity of prostaglandin E₂ (Bangbose and Noamesi, 1981) which is particularly believed to cause ovum release (Espey, 1980; Espey and Lipner, 1994b). The effect of cryptolepis in the present study was comparable to that of diclofenac suggesting that the mechanisms involved may be similar as both have potent anti-inflammatory action and interfere with prostaglandin activity.

Ovulation occurs 10 to 12 hours after mating in rabbits. When cryptolepis (62.5–500 mg/kg) was administered after ovulation, it had little effect on the ovulatory process; however uterine events were greatly affected. This was confirmed by the low implantation index and the high percentage of pre-implantation losses in animals treated with cryptolepis (Table 2). Cryptolepis particularly at 100 and 500 mg/kg had profound effects on the post ovulatory events compared to the reference anti-inflammatory agent diclofenac sodium. The number of corpora lutea was not significantly affected by treatment with cryptolepis, suggesting that reduction in ova release was not responsible for the difference in the number of implants. Together this suggests that cryptolepis could affect the process of fertilization, the rapid cell proliferation and differentiation from a zygote to embryo, decidualization and consequent implantation. Our previous report in mice (Ansah *et al.*, 2010) showed increased foetal sensitivity in mice between the periods of fertilization and implantation. Prostaglandins are important for processes from fertilization to implantation. This was demonstrated by Lim *et al.*

Table 2: Effects of cryptolepis on postovulatory events in female rabbits

Parameter	Diclofenac		Cryptolepis		
	Control	(3.3 mg/kg)	(62.5mg/kg)	(100mg/kg)	(500mg/kg)
Initial weight(kg)	2.34±0.15	2.34±0.07	2.30±0.11	2.08±0.14	2.44±0.06
Final weight (kg)	2.49±0.18	2.45±0.07	2.37±0.78	2.20±0.13	2.49±0.05
weight gain	0.15±0.03	0.11±0.03	0.07±0.04	0.12±0.05	0.05±0.04
No of corpora lutea	8.20±1.30	8.60±0.68	7.80±0.86	6.80±0.66	7.60±0.60
No of implants	6.60±1.14	5.80±0.37	4.00±0.71	2.0±0.71**†	2.4±0.51**†
Implantation index (%)	80	67	51	29	51
Preimplantation loss (%)	19.5	32	48.7	70	68

Implantation Index = number of implants x 100 / number of corpora lutea ; Results presented as mean±SEM. Statistical analysis is by one way ANOVA using Neuman-Keuls post hoc test. **means p<0.01. †means p< 0.05 compared with diclofenac. †† means p<0.01 when compared to diclofenac

(1997) where there were absolute implantation failure when blastocyst were transferred from wild type mice to COX-2 deficient mice. Notwithstanding the above mechanism, the enhanced sensitivity of the foetus particularly at high doses of cryptolepis coupled with the low implantation losses by diclofenac sodium suggest that other biological effects of cryptolepis which may not be possessed by diclofenac such as its cytotoxicity may be involved as cryptolepis and its main alkaloid, cryptolepine, cause cell cycle arrest by multiple mechanisms (Bonjean *et al.*, 1998; Lisgarten *et al.*, 2001; Ansah and Gooderham, 2002; Zhu *et al.*, 2005; Zhu and Gooderham, 2006; Ansah *et al.*, 2008; Ansah and Gooderham, 2009). This study shows clearly that cryptolepis treatment prior to ovulation leads to ovulatory failure and postovulatory treatment leads to reduced number of implants whilst increasing pre-implantation losses. The present findings together with our previous observation of inhibition of reproduction in cryptolepis-treated mice call for caution in the use of cryptolepis in females of child-bearing age.

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