CONTRACEPTIVE, ESTROGENIC AND ANTI-ESTROGENIC POTENTIALS OF METHANOLIC ROOT EXTRACT OF *CARPOLOBIA LUTEA* IN RODENTS

ETTE OKON ETTEBONG[1]*, PAUL ALOZIE NWAFOR[1], MEMFIN EKPO[2] AND KOLA KAYODE AJIBESIN[3]

[1]*Department of Pharmacology and Toxicology, Faculty of Pharmacy*
[2]*Department of Pathology, Faculty of Clinical Sciences, School of Health Sciences*
[3]*Department of Pharmacognosy and Herbal Medicine, Faculty of Pharmacy*
University of Uyo, P.M.B. 1017, Uyo, Nigeria

ABSTRACT

Several plants are used in herbal medicine for family planning. *Carpolobia lutea* is a medicinal plant in South Eastern Nigeria used for family planning. The study was designed to investigate the contraceptive, estrogenic and antiestrogenic potentials of the methanolic root extract of *Carpolobia lutea* in both rats and mice. The contraceptive effect of extract (7 - 21mg/kg) administered by intraperitoneal route for four days in divided doses was tested in mice and rats. Sexually-active males were introduced on day 5 at the ratio of 3F:1M and kept with these females till the end of the experiment. Investigations on the estrogenic and antiestrogenic property of the extract (7-21mg/kg) were done in immature rats that had undergone surgical removal of both ovaries. The effects of the extract (vaginal opening, vaginal cornification, uterine wet weight) were compared with 17-beta-estradiol (0.1µg/rat/day) as standard drug. Twenty-four hours later, the animals were sacrificed following the last dose and the weights of uterus, kidney, liver and small intestine were recorded. The extract prevented conception in both mice and rats for two gestational periods. Significant changes ($p<0.05$-0.001) were observed in the length and weight of pups relative to control. There were no abnormalities observed in the pups over thirty days. In ovariectomized immature young rats, the extract showed estrogenic effect (vaginal opening, vaginal cornification and increased uterine wet weight) in low doses while in high doses, it showed anti-estrogenic effect. These findings agree with the traditional use of *Carpolobia lutea* in the control of fertility. The contraceptive property of the extract may be associated with the direct effects of its chemical constituents.

Keywords: *Carpolobia lutea*; flavanoids, saponins, contraceptive activity, estrogenic.

INTRODUCTION

In the last decade, there has been a great upsurge in the pharmacological evaluation of medicinal plants especially in the third world that could be of benefit as contraceptive and fertility control agents and possible replacement for hormonal contraceptives. Many plants are known to have promising contraceptive properties (Farnsworth *et al.,* 1980).

Carpolobia lutea, G. Don (polygalaceae) is a medicinal plant that has been cited in traditional herbal medicine to facilitate delivery, treat male sexual disorders because of its aphrodisiac effect, helminthiasis and headache (Mitaine-Offer *et al,* 2002). It is a well known plant used in South Eastern Nigeria, especially among the Eket tribe as an aphrodisiac and contraceptive. Known popularly as cattle stick (English), Abekpok Ibuhu (Eket), Ikpafum, Ndiyan, Nyayanga (Ibibio), Agba or Angalagala (Igbo) and Egbo oshunshun (Yoruba), it is a small plant that often grows to 15ft in height having juicy fruits that the people of Southern Nigeria have found to be edible (Hutchinson and Dalziel, 1954). The plant is well distributed in West and also Central Africa (Mitaine-Offer *et al.,* 2002).

**Corresponding author:* e-mail: ettebong@yahoo.com

Carpolobia lutea has been known to have anti-arthritic and anti-inflammatory potentials (Iwu and Anyanwu, 1982), antimicrobial activities (Philips *et al.,* 2005) as well as antidiarrhoeal and anti-ulcerogenic properties (Nwafor and Bassey, 2007).

The present study has, therefore, been conducted to determine whether the root of this plant actually has any contraceptive, estrogenic and anti-estrogenic potentials.

MATERIALS AND METHODS

Plant material

Fresh root (1.7kg) of *C. lutea*, G. Don was collected in April, 2005 from Ekpene Obo in Akwa Ibom State, Nigeria. The plant was authenticated by Dr. (Mrs.) M. Bassey of the Department of Botany and Ecological Studies, University of Uyo and a voucher specimen with number UUH 126 was already assigned to it.

Extract preparation

The plant root (1kg) was oven-dried at 45°C, powdered and cold-macerated for 72h and extracted gradiently with 2.5 litre of n-hexane followed by chloroform, ethyl acetate and methanol to yield their respective extracts. These extracts were separately concentrated to dryness in vacuo at 40°C to give their respective dry extracts 16g

(1.6%), 10g (1.0%), 6g (0.6%) and 25g (2.5%) which were kept -4°C to be used when needed.

Phytochemical screening

Screening of the extract was done to determine the presence of chemical constituents such as flavonoids, simple sugar, alkaloids, tannins, saponins, phlobatannins, cardiac glycosides and anthraquinones following the method of Odebiyi and Sofowora (1978) and Trease and Evans (1989).

Animals

Adult and immature albino-type rats and mice with weights 180-220g, 60-90g and 20-26g respectively were obtained from the University of Jos, Jos, Plateau State and maintained strictly under favourable environmental conditions of 12 hr light/12hr dark cycle and temperature 22± 2.5°C and fed with growers pellet feed (Bendel Feeds and Flour Mills Ltd, Edo State) with water given as needed. Animal Ethics Committee, Faculty of Pharmacy, University of Uyo, gave the needed approval for the studies.

Studies of acute toxicity

To determine the LD_{50}, the albino mice were randomized and further divided into six groups consisting of three a group. They were fasted, only given water, 24hr before starting the experiment. The plant extract was given using the intraperitoneal (i.p) route and the dose range was 10 - 3,000 mg/kg. Signs of toxicity were noted in the animals for 24h. The method of Lorke (1983) was used to calculate the LD_{50}.

$$LD_{50} = \sqrt{ab}$$

a = dose producing 0% mortality
b = dose producing 100% mortality

Contraceptive activity

The contraceptive activity was determined using the method of Nwafor *et al.* (1998). Adult female mice and rats shown to have a regular estrus cycle through daily vaginal smear analysis and those having at least two successive 4-day estrus cycles were selected. The animals were randomized and separated into four groups consisting six animals per group. Normal saline (3ml/kg, mice; 5ml/kg, rats) was administered intraperitoneally in divided doses to the first group for 4 days. Groups two, three and four were administered with various doses of the extract 7, 14, and 21mg/kg intraperitoneally and respectively for a period of four days in divided doses.

Fertile and sexually-active males were introduced on day 5 in the ratio of 3:1 (F/M) and kept with the females till the end of the experiment. The number and weight of pups obtained after delivery were noted.

Anti-estrogenic and estrogenic potentials

For this investigation, both estrogenic and anti-estrogenic properties of methanolic extract were determined in immature rats after surgically removing both ovaries (Edgren and Calhoun, 1957; Nwafor *et al*., 1998). After one week, these young young immature animals three weeks old (weighing 70-90g) were randomized and separated into six groups each of which consisted of six animals.

Normal saline (5ml/kg) was administered to the first group for 4 days in divided doses. Groups two, three and four were administered with various doses of the plant extract (7, 14, and 21mg/kg) for 4 days respectively in divided doses. Group 5 received 17-beta-estradiol in corn oil administered through subcutaneous route (0.1µg/ rat per day) as standard drug successively for 4 days. Group 6 was administered with middle dose of extract (14mg/kg) and 17-beta-estradiol (0.1µg/rat daily) successively for 4 days to evaluate the antiestrogenic activity. The degree of vaginal opening and cornification in the animals were noted. Twenty-four hours after the last dose, the animals were sacrificed. Various weights of uterus, kidney, liver and small intestine were then recorded.

STATISTICAL ANALYSIS

Data obtained were analyzed using program Instat® and the results were expressed as multiple comparisons of Mean ± SEM. Significance was determined using one-way ANOVA and Tukey-Kramer multiple comparison post test. A p-value of less than 5% was taken as significant.

RESULT

Acute toxicity screening

The LD_{50} using Lorke's method, was 70.7mg/kg body weight. Excitation, gasping for breath, paw-licking, reduced movement, high respiratory rate, tonic-clonic convulsion and death were noted signs of acute toxicity.

Phytochemical screening

Screening of the methanolic plant extract indicated that the following chemical constituents were present: Saponins, Anthraquinones, Flavonoids, Cardiac glycosides, Terpenes and Simple sugar. However, tannins and alkaloids were absent.

Contraceptive activity

Table 1 shows the contraceptive activity of *Carpolobia lutea* extract in mice and rats. The methanolic extract protected mice from conception for two periods of gestation in 50% of animals previously treated with 21mg/kg to two periods of gestation in 80% of animals previously treated with 7mg/kg. Rats also had protection from conception for one period of gestation in 50% of animals previously treated with 21mg/kg to two periods

of gestation in 60% of animals previously treated with 7mg/kg.

Table 1: Contraceptive potentials of methanolic extract of *C. lutea* root in female mice and rats.

Dose of Extract (mg/kg)	Number of Pups Delivered	Degree of Protection over n-gestational period
Mice: Control	4.5 ± 1.09	Nil (0/6)
7mg/kg extract	1.33 ± 1.46	2(5/6) *
14mg/kg extract	2.00 ± 1.41	2(4/6) *
21mg/kg extract	2.33 ± 1.22	2(3/6) *
Rat: Control	5.17 ± 0.52	Nil (0/6)
7mg/kg extract	4.50 ± 0.71	2(4/6) *
14 mg/kg extract	4.00 ± 0.00	1(4/6) *
21mg/kg extract	3.33 ± 0.82	1(3/6) *

*Numerator indicates the number of mice that were protected.

However, this protection in mice and rats were reversible since after being allowed to mate, these animals became pregnant and bore pups at the expiration of the protected period without any foetal abnormality observed up to 30 days. The pups showed significant changes ($p<0.05$-0.001) in weights and lengths relative to control (tables 2 and 3).

Estrogenic and anti-estrogenic potentials of extract in young immature rats

The extract depicted a rise in the weight of uterus with increased dose peaking with the middle dose and falling with the highest dose. This initial increase was not significant but it resulted in vaginal opening and cornification observed with the lower doses and absent with high doses. But this increase in the weight of uterus could not be adequately reversed when 17-Beta estradiol was present (table 4).

DISCUSSION

These results show that the methanolic extract of *Carpolobia lutea* possesses contraceptive effect in mice and also in rats. They also depict estrogenic and anti-estrogenic potentials in rats. This is predicated upon the fact that animals previously treated with the extract and kept with sexually-active males were protected over some

Table 2: Contraceptive experiment in mice: The effect of methanolic extract of *C. lutea* on weight and length of pups

Treatment mg/kg	Day 1		Day 10		Day 20		Day 30	
	Weight (g)	Length (cm)	Weight (g)	Length (cm)	Weight (g)	Length (cm)	Weight (g)	Length (cm)
Control	1.58 ± 0.05	4.40 ± 0.05	5.15 ± 0.14	8.39 ± 0.08	8.58 ± 0.04	12.50 ± 0.02	12.27 ± 0.12	14.66 ± 0.17
7mg/kg	1.71 ± 0.02[a]	4.53 ± 0.05	4.70 ± 0.10[a]	9.01 ± 0.05[c]	11.22 ± 0.10[c]	13.95 ± 0.08[c]	16.06 ± 0.03[c]	16.65 ± 0.13[c]
14mg/kg	1.96 ± 0.02[c]	4.90 ± 0.03[c]	5.58 ± 0.12[a]	8.12 ± 0.03[a]	8.13 ± 0.19	12.30 ± 0.09	12.14 ± 0.54	14.48 ± 0.15
21mg/kg	1.31 ± 0.01[c]	4.01 ± 0.03[c]	3.96 ± 0.02[c]	7.60 ± 0.06[c]	6.16 ± 0.11[c]	10.55 ± 0.06[c]	9.11 ± 0.07[c]	12.62 ± 0.06[c]

Values show Mean ± SEM. Level of Significance relative to control [a]$p<0.05$; [b]$p<0.01$; [c]$p<0.001$; n=6

Table 3: Contraceptive experiment in rats: The effect of methanolic extract of *C. lutea* on weight and length of pups.

Treatment mg/kg	Day 1		Day 10		Day 20		Day 30	
	Weight (g)	Length (cm)	Weight (g)	Length (cm)	Weight (g)	Length (cm)	Weight (g)	Length (cm)
Control	5.96 ± 0.15	4.45 ± 0.12	13.68 ± 0.25	10.85 ± 0.09	20.39 ± 0.41	15.01 ± 0.25	33.00 ± 0.45	19.20 ± 0.18
7mg/kg	6.66 ± 0.05[b]	7.48 ± 0.06[b]	15.24 ± 0.54[a]	11.53 ± 0.10[c]	24.59 ± 0.31[b]	17.22 ± 0.16[b]	36.98 ± 0.45[b]	22.49 ± 0.17[b]
14mg/kg	5.81 ± 0.08	6.76 ± 0.09	13.88 ± 0.37	10.78 ± 0.12	22.91 ± 0.21[b]	15.99 ± 0.09[c]	33.92 ± 0.19	20.69 ± 0.13[b]
21mg/kg	5.16 ± 0,09[b]	5.98 ± 0.09[c]	9.86 ± 0.12[b]	9.52 ± 0.12[b]	12.17 ± 0.21[b]	12.56 ± 0.21[b]	16.58 ± 0.60[b]	15.64 ± 0.16[b]

Values show Mean ± SEM, Level of Significance relative to control [a]$p<0.05$; [b]$p<0.001$; [c]$p<0.01$; n=6

Table 4: Estrogenic and anti-estrogenic potentials of methanolic extract of *C. lutea* in rats

Group	Animal Weight	Dose Mg/Kg	Uterine Muscle Weight (g)	Kidney Weight (g)	Liver Weight (g)	Vaginal Opening	Vaginal Cornifi-cation
1.	74.17 ± 5.09	Control	0.05 ± 0.01	0.67 ± 0.006	3.43 ± 0.16	-	-
2.	86.83 ± 6.24	7mg/kg	0.05 ± 0.01	0.77 ± 0.04[a]	3.94 ± 0.17	2+	2+
3.	90.17 ± 6.04	14mg/kg	0.07 ± 0.01	0.85 ± 0.02[c]	4.54 ± 0.07[c]	+	+
4.	87.67 ± 5.83	21mg/kg	0.05 ± 0.01	0.66 ± 0.05	2.90 ± 0.12	-	-
5.	75.00 ± 3.94	17-β	0.41 ± 0.07[c]	0.74 ± 0.07	3.95 ± 0.1	5+	5+
6.	80.83 ± 2.42	14mg/kg+17-β	0.31 ± 0.04[c]	0.76 ± 0.03[a]	3.92 ± 0.09	3+	3+

Values Show Mean ± SEM. Level of Significance relative to control [a]$p < 0.05$; [b]$p < 0.01$; [c]$p < 0.001$ (n= 6).

varied gestational periods. There was an increase in uterine wet weight in low doses and a decrease in uterine weight in high doses which depicts a partial agonist-like effect. Moreso, premature vaginal opening and cornification were observed in young ovariectomized rats. Jacob *et al.,* (1969) demonstrated the uterotophic effects of estrogen when administered to rats and mice and Ljungkvist (1971) associated these effects with vaginal opening and cornification, endometrial growth and proliferation.

Estrogenic effects observed in premature animals is depicted by premature opening of vagina and vaginal cornification (Turner and Bagnara 1971). Steroidal saponin has been found to inhibit estrous cycle and reduce fertility in animals upon continuous administration (Tamura *et al.,* 1997). Ovulation is associated with inflammation (Epsey, 1980). Flavonoids are known to have anti-inflammatory effects which are believed to result from inhibition of cyclo-oxygenase enzyme (Liang *et al.,* 1999). The methanolic extract of *Carpolobia lutea* contains both saponins and flavonoids which may explain its contraceptive effects. The data from acute toxicity studies show that the extract is only moderately toxic. This moderate toxicity may have encouraged the local users in age-long use of the plant for family planning.

CONCLUSION

The data obtained demonstrated that the extract possessed estrogenic activity in low doses, which may contribute to its contraceptive effect. The reduced uterine wet weight at high doses suggests an anti-estrogenic effect and the reversal of the hormonal effect may indicate a non-hormonal mechanism or a direct effect of the chemical constituents of the extract.

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