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Uterotonic Properties of the Ethanol Extract of *Brysocarpus coccineus*

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Abstract

The ethanol extract of the leaves of *Brysocarpus coccineus* Schum. & Thorn. (Connaraceae) was investigated for uterotonic activity in non-gravid rat uteri. Repeated treatment with the extract enhanced spontaneous uterine muscle contraction. A marked increase was observed on day 14, which was sustained up to day 28. Furthermore, the extract increased the contractile force of the isolated myometrial preparation in a concentration-dependent manner. This effect was not attenuated by atropine, cyproheptadine or piroxicam, but was blocked by salbutamol, suggesting the involvement of β-adrenergic receptors. This result indicates the presence of biologically active substances with comparable effects to oxytocin on the uterus. The intraperitoneal LD_{50} in mice was found to be 547.7 ± 4.3 mg/kg. Preliminary phytochemical studies revealed the presence of carbohydrates, tannins, flavonoids and balsams.

Keywords: *Byrsocarpus coccineus*, rat uterus, adrenergic receptors, uterotonic activity.

Introduction

Brysocarpus coccineus Schum. & Thorn. (Connaraceae) is a shrub that is distinctively decorative and of great medicinal value in West Africa. It is widely used in ethnomedicine for the treatment of ear ache. In Ghana, the roots and a decoction of the leaves are applied to mouth sores as well as sores on the skin. The root bark, with black pepper (*Piper guineense*), is used to cure wounds. The plant is also used in the treatment of jaundice, tumors and inflammation, to arrest bleeding, swelling, pile, venereal disease and as an antidote for poisoning. In Nigeria, a cold infusion of the bruised leaves is used for the treatment of gonorrhoea (Dalziel, 1937; Irvine, 1961). The leaves have also been used traditionally in Northern Nigeria to induce labour and cause therapeutic abortion in Kogi State (Yakubu Habi – personal communication). From the available literature, no scientific report has been made on its oxytocic activity. In our effort to screen potential herbal medicines in Nigeria, we report in this paper the uterotonic activity of a 70% ethanol extract of the plant leaves. The oxytocic activity of the plant extract was evaluated using the isolated rat uterus and the effect on spontaneous contraction of the uteri following daily administration of the plant extract.

Materials and methods

Drugs

The drugs used in this present work were acetylcholine, atropine sulphate, oxytocin, ergometrine, indomethacin (Sigma Chemical Company, MO, USA), cyproheptadine (MSD, Lagos, Nigeria) and stilboestrol dipropionate (May and Baker, Dagenham, England).

Collection, identification and extraction

Leaves of *Brysocarpus coccineus* were collected from around Idu, Abuja, Nigeria. The plant was identified and authenticated by Mr. A. Oheari of the Department of Medicinal Plant Research and Traditional Medicine of the National Institute for Pharmaceutical Research and Development where a voucher specimen was deposited for future reference. The fresh leaves were washed clean with water, air-dried for 7 days and subsequently reduced to coarse powder using a mortar and pestle. The powder (83 g) was

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extracted in a Soxhlet apparatus using 70% alcohol for a period of 8 h. The extract was concentrated to dryness *in vacuo* under reduced pressure using a rotary evaporator to give a residue with a yield of 11.73% (w/w).

Phytochemical screening

Standard screening for various constituents, alkaloids, flavonoids, glycosides, etc., were carried out on the extract following the methods described by Trease and Evans (1983).

Acute toxicity studies (LD_{50})

The intraperitoneal (i.p.) acute toxicity (LD_{50}) study of the extract was carried out in Swiss albino mice as described by Miller and Tainter (1944). Briefly, the method involved administration of 5 different doses of the extract to 5 groups

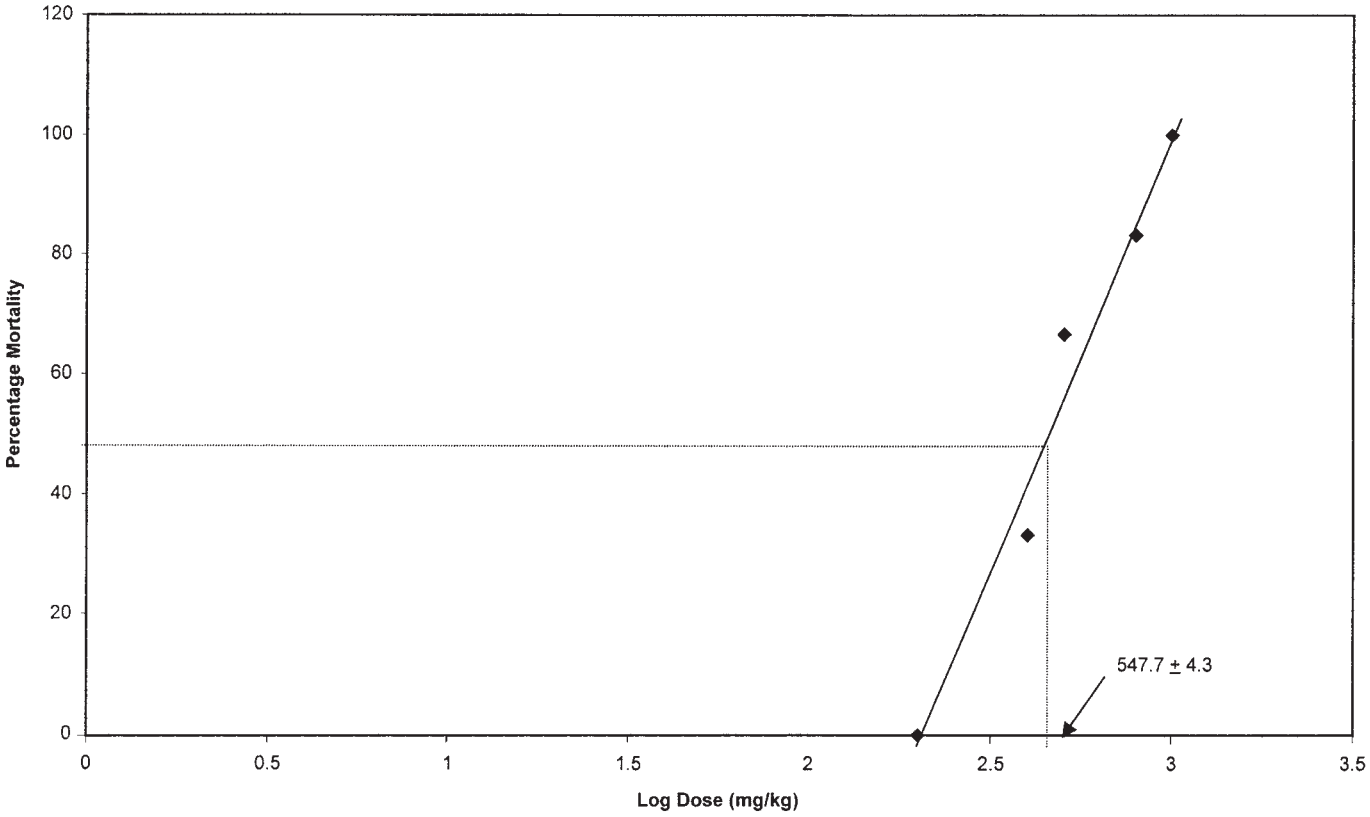

Figure 1. Graph showing the percentage mortality against log dose of the extract of *B. coccineus*.

Table 1. Phytochemical Screening of *Brysocarpus coccineus* leaf Extarct.

Tests	Observation	Inference
Sample + Molisch reagent + concentrated Sulphuric acid	Purple ring at interface	Carbohydrate present
Sample + Fehling's Solution A & B + heat	Brick red precipitate	Reducing sugars present
Sample + Ferric Chloride	Green colour	Tannins present
Sample + lead sub acetate	Precipitate formed	Tannins present
Sample + glacial acetic acid + ferric chloride, poured over concentrated sulphuric acid	No blue layer	Negative for digitalis glycoside
Sample + water + vigorous shaking	No froth formed	Negative for saponins
Sample + lead acetate	Yellow precipitate	Flavonoid present
Sample + amyl alcohol	Yellow colour	Flavonoid present
Sample + alcoholic ferric chloride	Dark green colour	Balsam present
Sample + Dragendorff's reagent	No precipitate	Negative for alkaloids
Sample + Picric Acid	No colour change	Negative for alkaloids

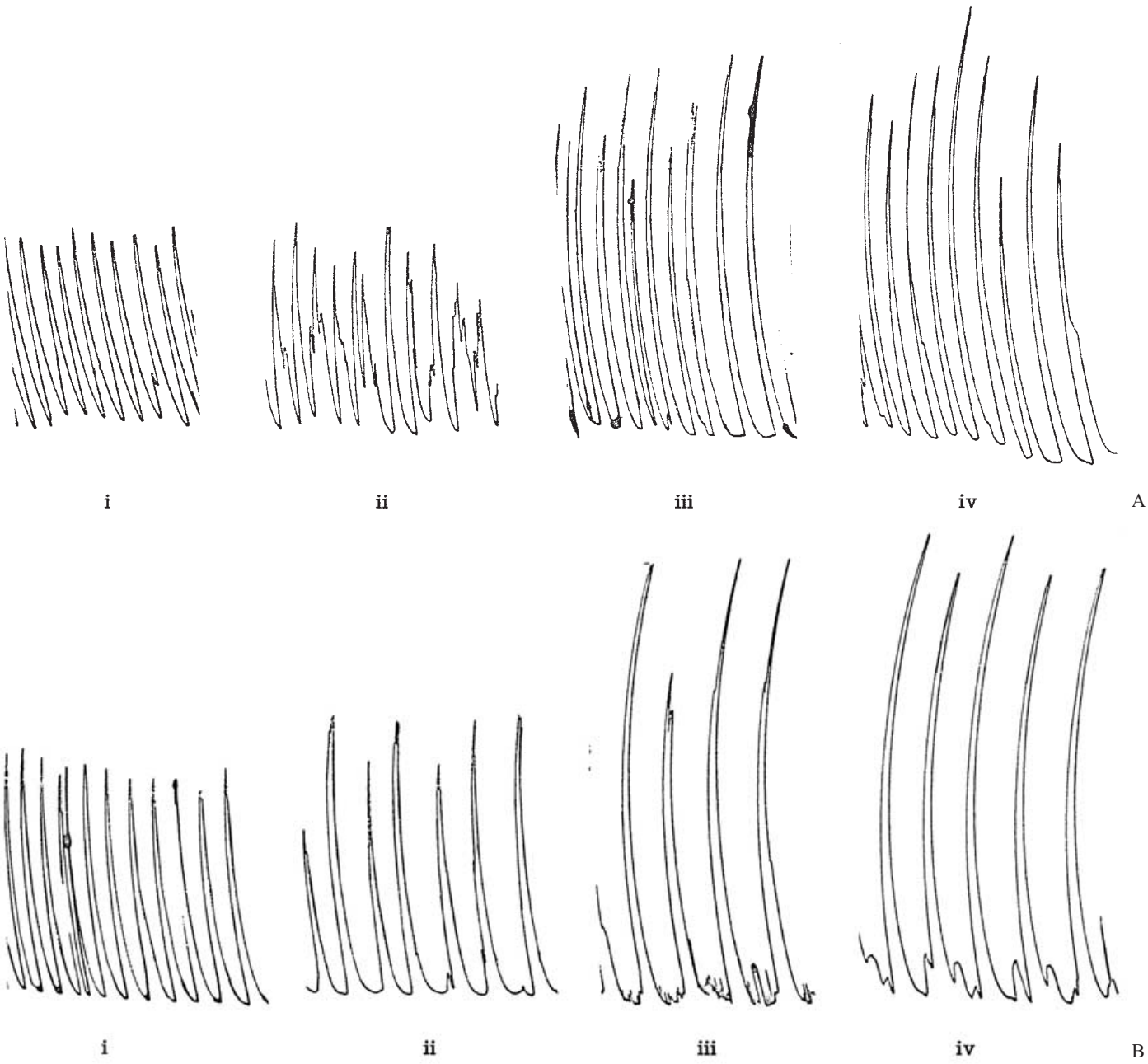

Figure 2. Typical tracings of the effect of ethanolic extract of *B. coccineus* (A: 25 mg/kg) and (B: 50 mg/kg) pretreatments (i) for day 0 (control); (ii) for 7 days; (iii) for 14 days and (iv) for 28 days on uterine contractions *in vivo*.

of mice (6 mice/group). The mortality in each group was recorded within 24 h. The (LD_{50}) was estimated from the graph of percentage (%) mortality (converted to probit) against log-dose of the extract probit 5 being 50%.

Experimental protocol

Daily doses of 25 and 50 mg/kg of the leaf extract were given orally to 2 groups of 10 female virgin Wistar rats weighing 100–120 g. A third group was given isotonic saline (0.9% NaCl). The rats were killed on day 0, 7, 14 and 28 by decapitation. The effect of repeated treatments on the spontaneous contractions of the uteri in De Jalon's solution aerated with air, maintained at a temperature of 37 ± 1 °C were recorded isometrically on a Ugo Basile Unirecorder 7050. A basal tension of 1 g was applied throughout the period of the experiment.

In another experiment, female Wistar rats weighing 150–180 g were pretreated with stilbestrol, 1 mg/kg subcutaneously, 24 h before the experiment. The rats were killed by

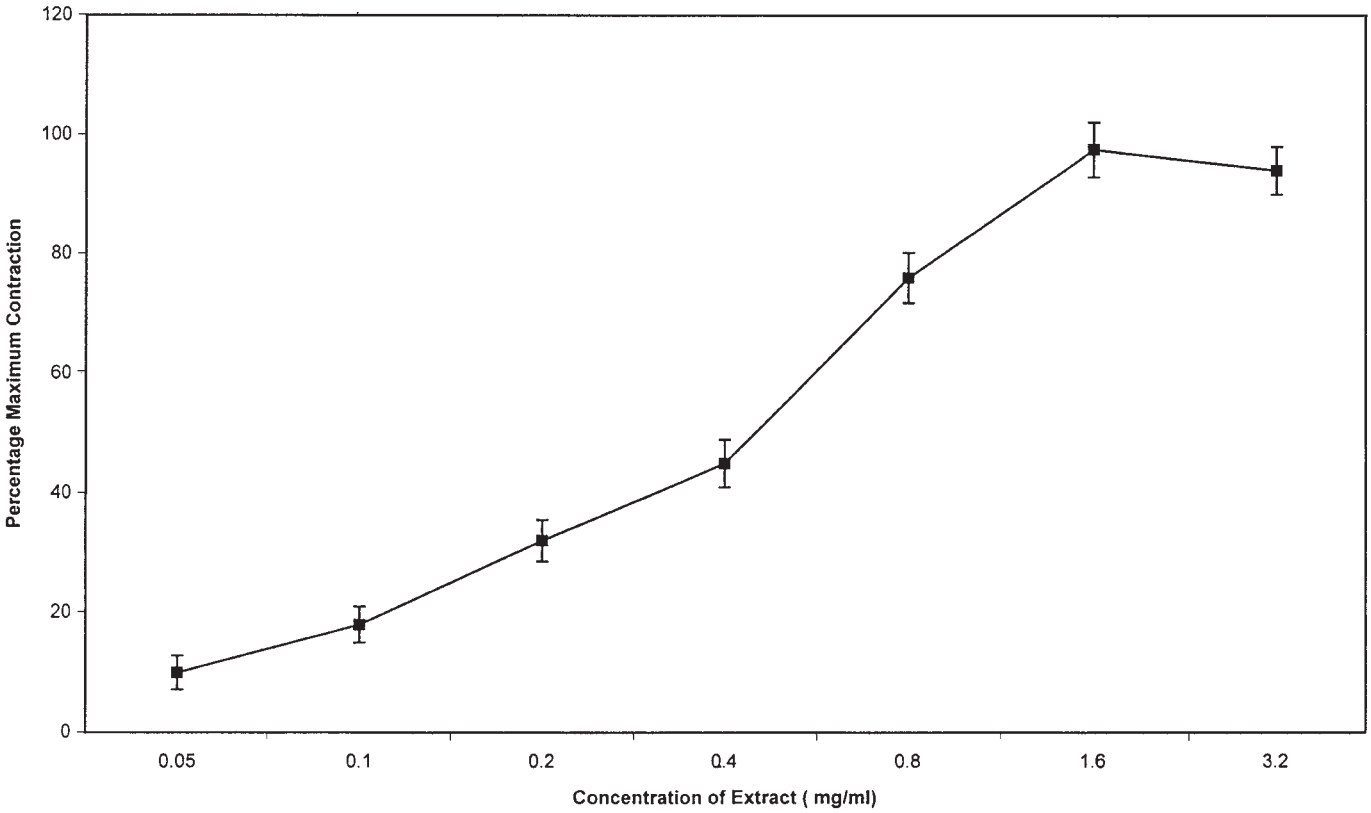

Figure 3. Concentration–dependent contraction of *B. coccineus* (0.05–3.2 mg/ml) on the isolated rat uterus.

cervical dislocation and exsanguinated. The uterus was isolated and the uterine horns dissected free of adhering tissues. A length of about 2 cm was obtained and mounted in a 20 ml organ bath containing De Jalon's solution of the following composition (mM): NaCl 154, KCl 5.63, $CaCl_2$ 0.41, $NaHCO_3$ 1.7, $MgCl_2$ 1.4 and D-glucose 5.5, maintained at 37 ± 1 °C and aerated with air. A tension of 1 g was applied throughout the experiment. The inhibitory effects of atropine (5×10^{-9} M), piroxicam (1×10^{-3} M) cyproheptadine (1×10^{-8} M) and salbutamol (2×10^{-8} M) on the extract – induced contraction of the rat uterus were evaluated. Furthermore, the effect of cyproheptadine (1×10^{-8} M) on ergometrine ($1.5 \times 10^{-5} - 1.2 \times 10^{-4}$ M) and 5-HT ($7.5 \times 10^{-4} - 5.6 \times 10^{-4}$ M) induced responses were investigated. The responses were recorded isometrically on an Ugo Basile Unirecorder 7050 through an isometric transducer 7004 after 1 h equilibration period.

Statistical analysis

Data obtained were expressed as mean ± SEM. The results obtained were analyzed statistically using the Student's *t*-test. The level of significance was $p < 0.05$.

Results and discussion

The intraperitoneal LD_{50} of the extract was found to be 547.7 ± 4.3 mg/kg (Fig. 1). Preliminary phytochemical screening gave positive reactions to carbohydrates, tannins, flavonoids and balsams (Table 1). Daily doses of 25 and 50 mg/kg of *B. coccineus* was found to enhance spontaneous uterine and muscle contraction of virgin female rats causing a graded increase in the force of contraction with respect to duration of treatment (Fig. 2). The extract produced an initial enhancement up to day 14 of treatment and this was sustained up to day 28.

On the isolated uterine preparations, the extract evoked a concentration-dependent increase in the amplitude of contractions (Fig. 3). This effect was similar to the findings of Uguru et al. (1998) on the effects of acetylcholine, oxytocin ergometrine and 5-HT on the rat uterus. Atropine (5×10^{-9} M), a known muscarinic blocker (Bolton, 1979), did not attenuate the contractions induced by the extract, but completely blocked the effects of acetylcholine, suggesting the lack of involvement of cholinergic receptors in the observed effects. Cyproheptadine (1×10^{-8} M), a 5-HT blocker (Hashimoto et al., 1977; Hartig, 1989), blocked the effect of 5-HT and ergometrine (Figs. 4A,B) but produced no effect on the extract induced contraction of the rat uterus, indicating that 5-HT receptors might not be involved in the observed effect. Piroxicam (1×10^{-3} M) is one of the most widely used non-steroidal anti-inflammatory drugs which produce their actions primarily through the inhibition of prostaglandin biosynthesis (Vane, 1971; Gamaniel et al., 1988). The drug did not attenuate extract induced contraction of the uterus.

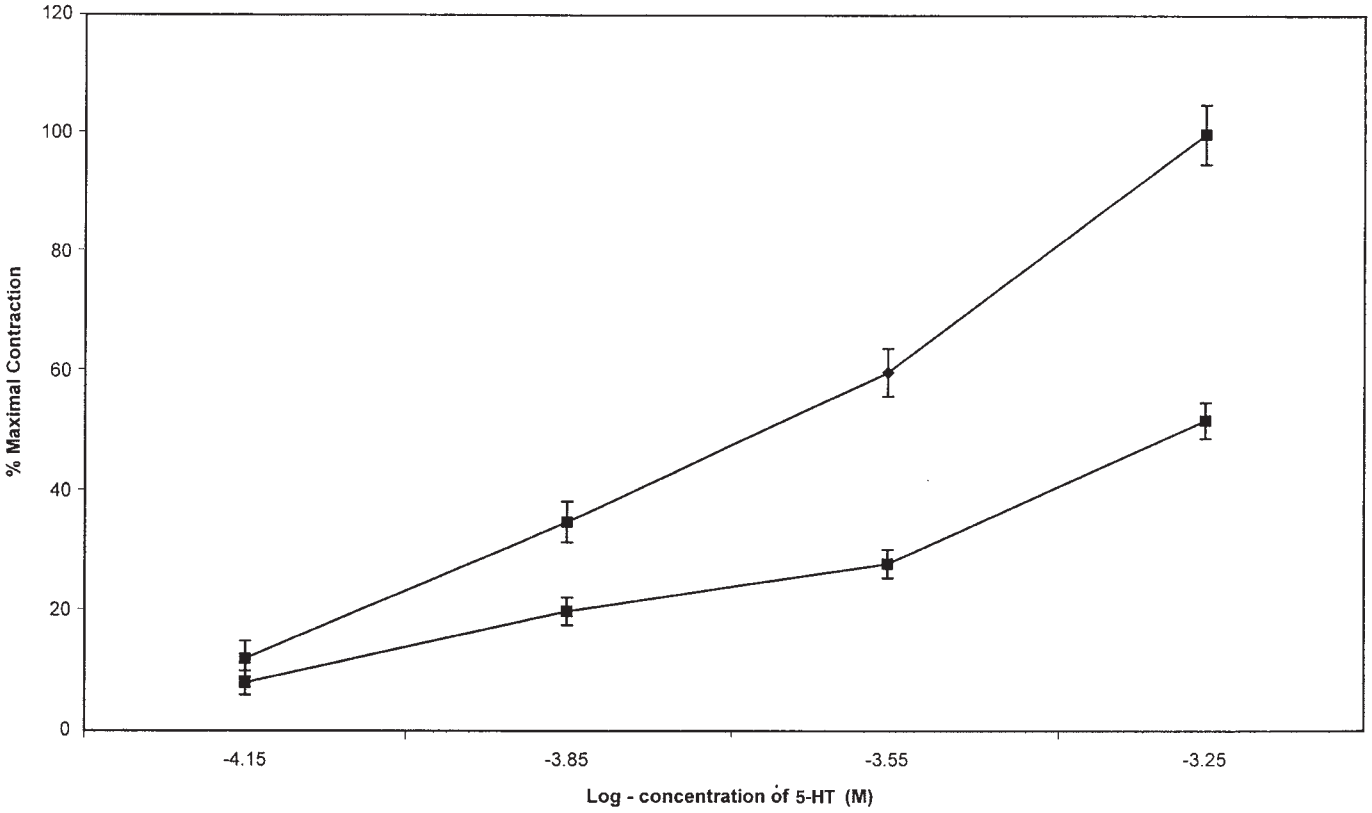

Figure 4. Inhibitory effects of cyproheptadine (■, 1×10^{-8} M) on (A) 5-HT (◆, $7.5 \times 10^{-4} - 5.6 \times 10^{-4}$ M) and (B) ergometrine (▲, $1.5 \times 10^{-5} - 1.2 \times 10^{-4}$ M).

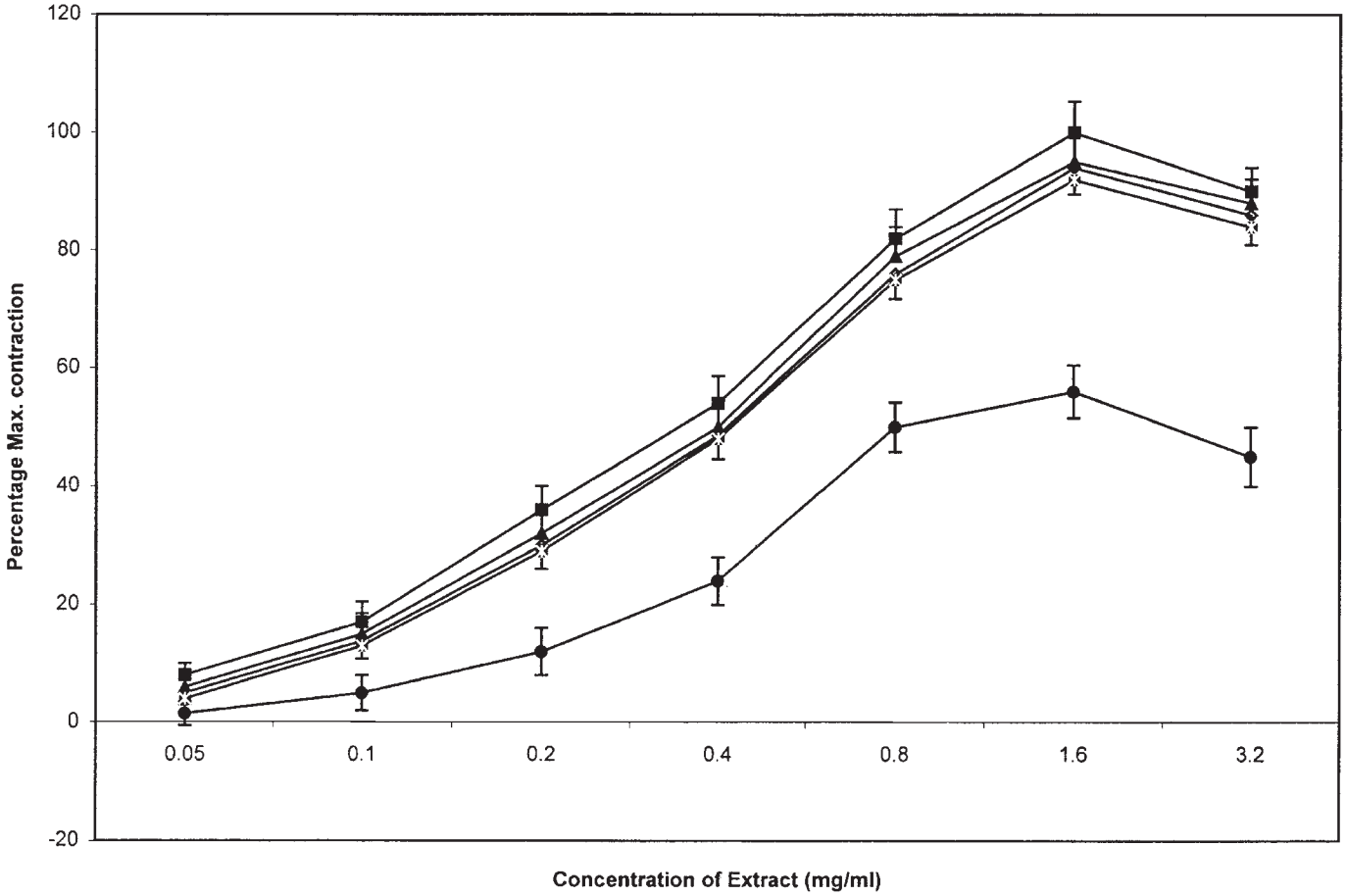

Figure 5. Contraction response curves to ethanolic extract of *B. coccineus* alone (■) and in the presence of (▲) atropine 5×10^{-9} M, (◇) piroxicam 1×10^{-3} M, (✳) cyproheptadine 1×10^{-8} M and (●) salbutamol 2×10^{-8} M.

The response observed with both oxytocin and the extract were abolished by the β_2 agonist salbutamol (Fig. 5) These effects might have been achieved via physiological antagonism (Rang et al., 1995). The extract and salbutamol may therefore physiologically antagonize the effects of each other similar to oxytocin. Work is ongoing in our laboratory to isolate and characterize the active compound with possible application in humans.

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