

Original article

RELAXANT EFFECTS OF THE AQUEOUS LEAF EXTRACT OF *CASSIA OCCIDENTALIS* ON RAT AORTIC RINGS

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The relaxant effects of an aqueous extract of the leaf of plant cassia occidentalis (C.O.) were investigated in rat aortic rings with or without intact endothelium. The extract inhibited contraction elicited by noradrenaline (NA) and Potassium Chloride (KCl) dose dependently. It also relaxed aortic rings precontracted with 10⁻⁷ M NA and 50m M KCl. This relaxation did not require the presence of an intact vascular endothelium and was not affected by indomethacin (Prostacylin inhibitor) and methylene blue.

Key words: *Cassia occidentalis*; Rat aorta; Vasodilatation, Vascular endothelium.

INTRODUCTION

The worldwide increasing demand for medicine from natural sources (Lapa, 1992) has motivated us to search for plants with potential hypotensive activity. The leaf of *cassia occidentalis*, family Caesalipniaceae is a small tree growing 5 to 8 metres in height. The leaf of this plant is used in local folk medicine as an antihypertensive agent (Oliver, 1960; Sadique, 1987). However, we have found no scientific prove to the claims ascribed to this plant.

Hypertension is a significant health problem, because of the percentage of the population affected and the serious consequences of uncontrolled high blood pressure (Gerber and Neis 1990). It is also known that agent that lowers blood pressure may do so by relaxing vascular smooth muscle (Ajagbonna et al 1995:1999). In this preliminary study, the possible vascular smooth muscle relaxant effect of an extract of *cassia occidentalis* was studied in vitro in aortic rings.

MATERIALS AND METHODS

Plant Materials:

The plant was collected within Sokoto metropolis in Nigeria and authenticated by a traditional medical practitioner and the Department of Botany of Usmanu Danfodiyo University, Sokoto, Nigeria. A voucher specimen is kept in the herbarium for reference purposes.

The leaves were air-dried at room temperature for a week, crushing of the dried leaves into powder was done using a pestle and mortar. 40g of the powdered leaves were weighed into a conical flask containing 400ml of distilled water; the mixture was shaken and allowed to stand for 30 minutes. It was then boiled for one hour, cooled as shaken before filtration using a dry Whatman filter paper into a measuring cylinder. The filtrate was then concentrated by evaporation in a water bath and stored at 4°C until used.

Tissue Preparation:

Inbred adult male and female Sprague Dawley rats weighing 150-170g were obtained from the laboratory animal centre of the Faculty of Veterinary Medicine

Usmanu Danfodiyo University, Sokoto. The rats were sacrificed by cervical dislocation. The thoracic aorta was quickly removed, freed of connective tissue and placed in a petri-dish containing physiological salt solution (PSS) consisting of 119m M NaCl; 4.7m M KCl; 1.2m M KH₂PO₄; 1.2m M MgSO₄; 15.0m M NaHCO₂; 1.5m M CaCl₂; and 11.5m M glucose. The aorta was cut into 2mm rings and mounted in 20ml jacketed tissue baths containing PSS.

The PSS was continuously bubbled with 95 percent O₂ and 5 percent CO₂ gas mixture with the pH maintained at 7.4 using a pH meter model 305L (N. Boyer, UK). The bath temperature was kept at 37°C by continuous circulation of water around the bath from a thermostatically controlled water bath (Grass Instrument Ltd) using a roller pump (Watson-Marlow, Ltd). The aortic ring was mounted on fine stainless steel rod which was connected to a grass force transducer (Grass medical instrument, Quincy, Mass model FTO3) for the measurement of changes in isometric contraction. The force transducer was connected to a Grass model 7 polygraph for the display of recordings.

The ring was under an initial tension of 2g and was allowed to equilibrate for a period of 90 min. during which tissue was stimulated 3 times with a stabiliser dose of either 10⁻⁷M noradrenaline (NA) or 50m M Potassium Chloride (KCl) to obtain a reproducible response. After a stable response must have been achieved, the following in vitro protocol were carried out:

Concentration response of Aortic Rings to *Cassia occidentalis*:

The effects of *cassia occidentalis* on base line was determined after which the ring was contracted using 10⁻⁷M NA or 50m M KCl. After the contraction of the ring had stabilised, *cassia occidentalis* extract (1-8mg/ml) were added cumulatively into bath solution. The effect of each concentration was allowed to reach a steady level before the addition of the next dose.

Concentration responses to NA and KCl:

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Some aortic rings were exposed to cumulative concentrations of NA (10^{-9} to 10^{-5} M) and KCl (10 to 100m mol). Later on, the aortic rings were incubated in PSS containing either 1.8mg/ml or 3.6mg/ml *cassia occidentalis* for 10 minutes and thereafter the cumulative response to NA and KCl were repeated.

Endothelium Mechanisms:

Some aortic rings without intact endothelium were also contracted with 10^{-7} M NA and relaxation responses to cumulative concentrations of *cassia occidentalis* was also repeated. The endothelium was removed by gently rubbing the inner lining of the rings with a fine platinum wire and complete removal of the endothelium was confirmed by the absence of a relaxation response to NA pre-contracted rings to 10^{-5} M acetylcholine (Ajagbonna and Sofola 1999).

In some other experiments, the influence of 10^{-6} M methylene blue (MB), a guanylate cyclase inhibitor on the relation responses induced by *cassia occidentalis* was examined by pre-incubating the tissue with methylene blue for 15mins incubation period, the aortic rings were stimulated with 10^{-7} M NA before observing responses to varying concentrations of *cassia occidentalis* extract.

In other experiments, the rings were incubated in physiological salt solution containing 10^{-5} M indomethacin, an inhibitor of prostacyclin for 15min and then subjected to a concentration response test to *cassia occidentalis* in rings stimulated with 10^{-7} M NA.

Values are expressed as a percentage of the maximum response to 10^{-9} M NA.

Statistics

Values are expressed as Mean \pm SEM. Significant differences among the treatments were determined by analysis of variance (ANOVA). The IC 50 (concentration of the extract required to produce 50% of maximal relaxation were determined using a programme for logit transformation of concentration response curves.

RESULTS

Relaxant effects of C.O on aortic rings pre-contracted with NA and KCl.

Fig 1a and b show the effect of C.O extract on NA and KCl pre-contracted rings. Treatment of the ring with NA and KCl resulted in contraction of the rings. C.O. was observed to produce a concentration dependent relaxation of the pre-contracted rings (see Table 1).

Table 1;

Relaxation response to *cassia occidentalis* in aortic rings precontracted with 10^{-7} M NA with (+E) or without (-E) endothelium, rings treated with methylene blue (MB) and indomethacin.

| Contractile agent | IC ₅₀ | % maximum relaxation |
|-------------------------------------|------------------|----------------------|
| 10 ⁻⁷ NA (+E) | 3.07 \pm 0.5 | 98 \pm 5.2 |
| 10 ⁻⁷ NA (-E) | 2.9 \pm 0.3 | 90 \pm 4.5 |
| 10 ⁻⁷ NA (+MB) | 3.1 \pm 0.6 | 96 \pm 3.6 |
| 10 ⁻⁷ NA (+Indomethacin) | 3.2 \pm 0.7 | 95 \pm 5.5 |

The extract at 8mg/ml produced a maximum relaxation of $98 \pm 1.8\%$ in NA pre-contracted rings and $80 \pm 5.2\%$ relaxation in KCl pre-contracted rings. (Figs. 1a and 1b). The IC₅₀ was 3.07 ± 0.57 mg/ml in NA pre-contracted rings while it was 3.63 ± 0.85 mg/ml in KCl pre-contracted rings.

Effect C.O on Contractile Responses to NA & KCl

Cumulative addition of NA and KCl to isolated aortic rings resulted in concentration dependent contraction of the rings. Incubation of aorta in PSS containing *cassia* extract significantly reduced the contractile responses to NA and KCl in a concentration dependent manner with a shift of the contraction response curve to the right (Figs 2 and 3).

Effect of C.O on Endothelium Mechanism

Removal of endothelin (-E), treatment with methylene blue (MB) and indomethacin did not significantly ($P > 0.05$) affect maximum tension developed when the different rings were pre-contracted with 10^{-7} M NA. (Data not shown). The maximal relaxation produced with C.O. extract in the indomethacin treated rings, MB treated and in rings without endothelium were comparable to that of the control rings. The IC₅₀ was also not significantly ($P >$

0.05) different in the various rings when compared to the control.

DISCUSSION

The results from this study indicate that the aqueous extract of leaf of *cassia occidentalis* possess relaxant effect in rings of rat aorta with or without intact endothelium.

A number of agents have been shown to relax vascular smooth muscle through the release from the endothelium a labile relaxing factor, endothelium dependent relaxing factor (EDRF) (Furchott and Vanhoutte 1989; Ajagbonna and Sofola 1999) and methylene blue prevents the formation by inhibiting guanylate cyclase.

Methylene blue in this study did not alter the cassia-induced relaxation (Table 1) suggesting non- involvement of endothelium in its relaxation. Also, indomethacin, a selective prostacyclin inhibitor did not alter the dilator influence of prostacyclin, this also suggest that relaxation response of cassia extract may not be associated with a prostacyclin mediated mechanism.

Contraction of smooth muscle is brought about by an increase in the intracellular concentration of calcium (Bohr 1973; Ajagbonna et al 1995). Such an increase is possible by the influx of this ion through two distinct types of specific channels (Bolton 1979). Channels sensitive to voltage changes are activated by depolarizing agents such as KCl whereas those operated by receptors mediate responses to specific agonists e.g noradrenaline (Adegunloye et al 1993, Ajagbonna et al 1995). Receptor activation also leads to increased cytoplasmic calcium concentration by release of the ion from intracellular stores (Somlyo 1985; Ajagbonna et al 1996). It is therefore

thought that smooth muscle relaxation is related to both inhibition of calcium influx and intracellular release.

The fact that *cassia occidentalis* extract antagonized and relaxed contraction induced by receptor agonist (NA) and KCl suggest that cassia extract may be relaxing smooth muscle by inhibiting Ca^{2+} influx through receptor operated channel and voltage sensitive channel showing therefore its non specificity on these Ca^{2+} channels.

In summary, our results in this study show that the hypertensive activity of *Cassia occidentalis* extract may be by direct relaxant effect which may therefore justify its extensive use in folk medicine as an antihypertensive agent.

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