EFFECTS OF *TETRAPLEURA TETRAPTERA* (TAUB) FRUIT EXTRACT ON SOME ISOLATED TISSUES: POSSIBLE MECHANISM(S) OF ANTIHYPERTENSIVE ACTION

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ABSTRACT

Hypotensive activities of the alcoholic extract of T. tetraptera fruits have been earlier demonstrated in cats, rats and rabbits. In the present study, we demonstrate myorelaxant actions of the extract in isolated smooth muscle preparations. Relaxations were dose-dependent: EC_{50}s were 0.3 ± 0.01, 2.4 ± 0.1 and 4.3 ± 0.01 mg/ml for rat anococcygeus, rat uterus and guinea-pig taenia coli respectively. Also, spasmogenic actions of noradrenaline, carbachol, Ca^{2+} and K^+ in the rat anococcygeus and uterus were all suppressed by the extract. The effect of the extract on Ca^{2+}-induced contractions were investigated in guinea-pig isolated taenia coli and atria preparations and compared to nifedipine, a calcium-channel blocker . Pre-treatment with T. tetraptera (1-10 mg/ml) and nifedipine (0.01-0.1 µg/ml) for 15 minutes in Ca^{2+}-free K^+ (100 mM) medium caused a right ward shift of the concentration-response curves for $CaCl_2$-induced contractions in atria and taenia coli with depressed maximal response, suggestive of a non-competitive interaction. In all tissues investigated, the extract non-specifically reduced both the natural tone and agonist-induced contractions. Thus smooth muscle relaxant actions of the extract may partly account for the hypotensive actions of T. tetraptera fruits.

INTRODUCTION

Hypertension, a major risk factor for cardiovascular and renal morbidity and mortality, is one of the leading contributors to global disease burden (4.5%) and is as prevalent in many developing countries, as in the developed world (Murray and Lopez, 1997; Whitworth, 2003).

The prevalence of hypertension in Ghana is 29-30% (Cappuccio *et al*., 2004; Agyemang *et al*., 2006) and it accounts together with renal failure for 28.5% of mortalities (Plange-Rhule *et al*., 1999). In Ghana, a large percentage of the population relies on herbal medicines as alternatives to orthodox medicines probably due the lack of such medicines or for economic reasons (WHO, 2003; Ohene Buabeng *et al*., 2004). There is therefore the urgency for further research to ascertain the efficacy and safety of several other practices and medicinal plants (WHO, 2003).

In Ghana, several herbal medicines including *Tetrapleura tetraptera* (prekese) are used in the Ashanti Region to manage hypertension (Abel and

Busia, 2005). We have recently demonstrated the antihypertensive effect of a hydroalcholic extract of *T. tetraptera*, in cats, rats and rabbits (Amissah *et al*., 2007), thus confirming earlier reports by other workers (Ojewole and Adesina, 1983; Adewunmi, 2001). We also found that the actions of the extract precluded histaminergic, muscarinic and beta adrenergic involvement (Amissah *et al.* 2007). Furthermore, results from work on the frog heart, guinea-pig atria and rabbit aortic strip were suggestive of inhibition of calcium activity. The present studies investigate the effect of *T. tetraptera* on some isolated smooth muscle and atria preparations to further elucidate the possible mechanism(s) involved in the previously observed cardiovascular effects. These studies have been performed on rat anococcygeus, rat uterus, guinea-pig taenia coli and guinea-pig atria.

MATERIALS AND METHODS

Plant material

Fruits of *T. tetraptera* were obtained from Centre for Biodiversity Utilization Development (CBUD), KNUST, Kumasi, where it had been authenticated.

Preparation of ethanolic extract

The fruits were air-dried and powdered in a hammer-mill. The powdered fruits (1 kg) were soxhlet -extracted using 70% ethanol over a period of 21 hours. The resulting extract was concentrated under reduced pressure and a temperature at 60 ^{o}C to a syrupy mass. It was then dried to a dark brown semi-solid mass and kept in a dessicator till it was used. Final yield was 299.98 g of dry extract (percentage yield being 30 % $^{w}/_{w}$). This is subsequently referred to as TTE or the extract.

Animals

Sprague-Dawley rats (150–300 g), and guinea-pigs (350-400 g) of either sex fed on normal commercial pellet diet (GAFCO, Tema) and given water *ad libitum* were used in these studies. All animals were maintained under standard conditions of light, temperature and humidity. Frogs used were obtained from ponds on the KNUST Campus, Kumasi.

Drugs

Acetylcholine, potassium chloride, and calcium chloride were obtained from BDH Chemicals Ltd (Poole, England), isoprenaline, noradrenaline, phentolamine, and atropine from Sigma Chemicals Company (St Louis, MO, USA), stilboesterol from Abbott (Japan) and nifedipine from KRKA Pharmaceuticals (Slovenia).

Rat anococcygeus muscle

Anococcygeus muscles from male Sprague-Dawley rats were dissected out according to the method of Gillespie (1972). The tissues were mounted in a 10 ml isolated organ bath containing Krebs' physiological solution of the following composition (mM): NaCl, 155.0; KCl, 0.58; $NaHCO_3$, 0.6; $CaCl_2$, 0.1; and glucose, 2.5. The bathing medium was continuously gassed with 95% O_2/CO_2 and temperature was maintained at 37^{o}C and constantly aerated. Resting tension of 0.5 g was applied and the tension developed was measured with Harvard force displacement transducer (type A-6360) coupled to Harvard Universal oscillograph (type 50-8622). Preparations were allowed to equilibrate for 30 minutes before starting the experiment.

Cumulative dose–response curves to noradrenaline, Ca^{2+}, K^{+}, and carbachol were obtained in the absence and presence of TTE (1–10 mg/ml).

Rat Uterus Preparation

Female non-pregnant Sprague-Dawley rat was injected 24 hours before the experiment with 0.1 mg/kg of stilboesterol in order to induce oestrus (Estañ *et al.,* 1988). The rat was stunned with a sharp blow at the head and exsanguinated. The uterine horns were dissected out and each tube cut longitudinally so that the preparation was a sheet of muscle. The sheet of muscle was mounted in a 10 ml organ bath containing De Jalon's physiological solution of the following composition (mM): NaCl, 155.0; KCl, 0.58; $NaHCO_3$, 0.6; $CaCl_2$, 0.1; and glucose, 2.5. The bathing medium was continuously gassed with 95% O_2/CO_2 and temperature was maintained at 32 ^{o}C. Resting ten-

sion of 1 g was applied and the tension developed was measured with Harvard force displacement transducer (type A-6360) coupled to Harvard Universal oscillograph (type 50-8622). The tissues were allowed to equilibrate for 30 minutes before starting the experiment.

Cumulative dose-response curves were obtained for effect of TTE on the preparation. Cumulative dose–response curves to acetylcholine before and in the presence of atropine (0.005–0.5 µg/ml) and TTE (0.1–10 mg/ml) were obtained. Cumulative dose–response curves were also obtained for K^+ in the presence of TTE (0.1–10 mg/ml).

Guinea-pig taenia coli

Strips of taenia coli isolated from the caecum of guinea-pigs were immersed in modified Tyrode physiological solution of following composition (mM): NaCl, 155.0; KCl, 5.7; $NaHCO_3$, 6.0; $CaCl_2$, 1.0; and glucose, 5.5. The bathing medium was constantly aerated and maintained at 37 ^oC. The muscle strips were suspended in 10 ml organ bath under resting tension of 0.5 g and allowed to equilibrate for 40 minutes with several changes of the Tyrode physiological solution. Contractions were recorded with Harvard force displacement transducer (type A-6360) coupled to Harvard Universal oscillograph (type 50-8622).

Cumulative dose-response tracings were obtained for TTE and noradrenaline. In another experiment to investigate the effect of the extract on calcium-induced contractions, the muscles were suspended in Ca^{2+}-free solution for 30 minutes with several changes of the solution and thereafter in Ca^{2+}-free, isotonic 100 mM K^+ solution for 15 minutes. Dose-response curves of $CaCl_2$ were obtained by cumulative addition in the absence and in the presence of TTE (1-10 mg/ml) and nifedipine (0.01-0.1 µg/ml). The drugs tested were added to the bath 15 minutes before cumulative addition of $CaCl_2$. The effects were expressed as percentages of the maximum response of the $CaCl_2$-induced contractions.

Guinea-pig atria

Effects of *T. tetraptera* and nifedipine on the contractions of partially-depolarized atria were studied using a modification of method described by Nabata (1977). Briefly, guinea pigs were stunned with a blow to the head and the atria were isolated and suspended in a 10-ml tissue bath containing Krebs-Henseleit solution of the following composition (mM): NaCl, 118.4; KCl, 4.7; $NaHCO_3$, 24.9; $CaCl_2$, 2.5; KH_2PO_4, 1.2; $MgSO_4$, 1.2; and glucose, 11.1. The bathing medium was constantly aerated with 95% O_2/CO_2 and maintained at 37 ^oC. Contractions were recorded by means of a force-displacement transducer (model A-6360; Harvard Apparatus Ltd, Kent, England) coupled to an oscillograph (model 50-8622, Harvard Apparatus Ltd, Kent, England). The resting tension was adjusted to 1.0-1.5 g and the tissues were allowed to equilibrate for 30 min until a stable atrial rate was obtained.

After equilibration period, the bathing Krebs-Henseleit solution was replaced with a high K^+-low Ca^{2+} solution. Isoprenaline (1 µg/ml) was added to the bathing solution to induce maximum contractions. Dose-response curves of $CaCl_2$ were determined by cumulative addition to the bath in the absence and in the presence of *T. tetraptera* (0.5 mg/ml) and nifedipine (0.5 µg/ml). The drugs tested were added to the bath 30 minutes before cumulative addition of $CaCl_2$ in the presence of isoprenaline (1 µg/ml). The effects were expressed as percentages of the control maximum $CaCl_2$-induced contraction

Analysis of Data

For the determination of ED_{50} (dose responsible for 50% of the maximal effect), inhibitory effects of drugs were analyzed by using an iterative computer least squares method using GraphPad Prism for Windows version 4.02 (GraphPad Software, San Diego, CA, USA), with the following nonlinear regression (four-parameter logistic equation):

$$Y = \frac{a + (a - b)}{1 + 10^{((Log\ \ ED_{50} - X) \times Hill\ \ Slope)}}$$

Where, *X* is the logarithm of concentration. *Y* is the response and starts at *a* and goes to *b* with a sigmoid shape.

Statistical analyses were by one-way ANOVA followed by Student-Newman-Keuls or Tukey-Kramer's post test using GraphPad Prism.

RESULTS AND DISCUSSION

Relaxation effect was observed with cumulative addition of *T. tetraptera* on all the smooth muscle preparations (Fig. 1). This may be attributable to compounds such as tannic acid present in the plant shown to inhibit contractions of isolated rat uterus (Joao *et al.*, 1986). Furthermore, scopoletin which has been isolated from this plant is a non specific spasmolytic agent (Ojewole, 1983).

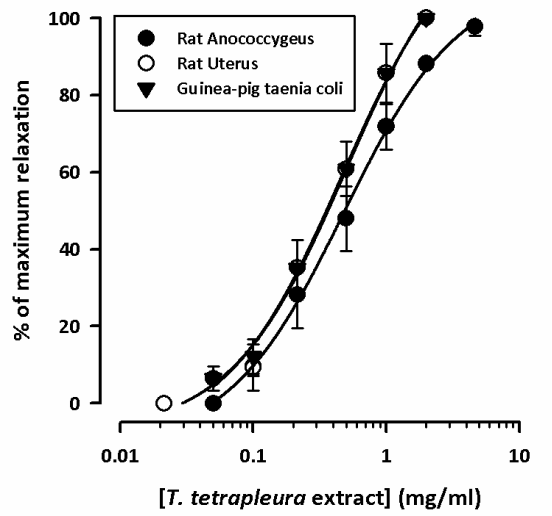

Fig. 1: Concentration-response curves of *T. tetraptera* on isolated rat anococcygeus, rat uterus and taenia coli preparations. Each point represents the mean ± S.E.M. (n = 3). EC_{50} (mg/ml) values of *T. tetraptera* on rat anococcygeus 0.4795 ± 1.12; rat uterus 0.4995 ± 1.35; taenia coli 0.4609 ± 1.41. *Note:* graphs for rat uterus and guinea-pig taenia coli are identical.

Isolated Rat Anococcygeus Muscle Preparation

T. tetraptera (0.5-10 mg/ml) produced concentration-dependent relaxations in rat anococcygeus (Fig. 1). Pre-contractions induced by KCl, noradrenaline or carbachol were relaxed by *T. tetraptera* concentration-dependently (Fig. 2).

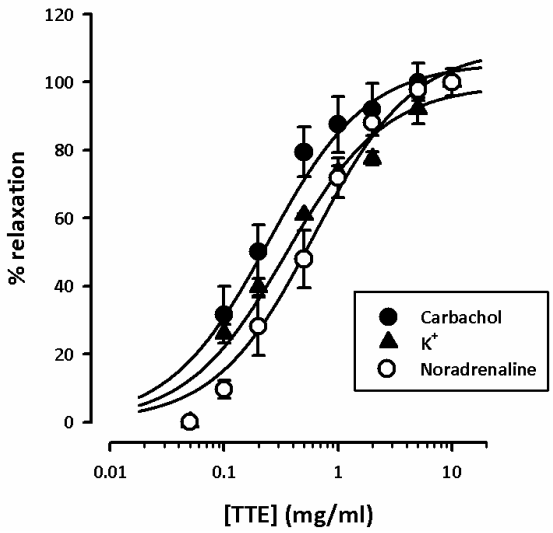

Fig. 2: Concentration-response curves of *T. tetraptera* on isolated rat anococcygeus preparation pre-contracted with carbachol, noradrenaline and K^+. Relaxations are expressed as percentages of maximum contraction produced by noradrenaline. Each point represents the mean ± S.E.M. (n = 3). EC_{50} (µg/ml) values of *T. tetraptera* for ; noradrenaline-induced curve, 488.65 ± 0.12; carbachol-induced curve, 35.89 ± 120.80; K^+ -induced curve, 23.04 ± 6.39.

Pre- treatment with *T. tetraptera* (1-10 mg/ml) for one minute caused a rightward shift of concentration-response curves of noradrenaline, carbachol, $CaCl_2$ and KCl. (Fig. 3). The EC_{50} and percentage inhibition values are as shown in Table 1. In all instances, the antagonism was non-surmountable. This therefore suggests that the antagonism may be irreversible competitive or noncompetitive. In the latter scenario, antagonism describes the situation where the antagonist blocks at some point the chain of events that leads to the production of a response by the agonist. Since *T. tetraptera* blocked non-specifically the contraction of anococcygeus produced by all the test agonists, it was speculated that it could act by preventing the influx of calcium ions through the cell membrane. In support of this, Ojewole (1983) has reported

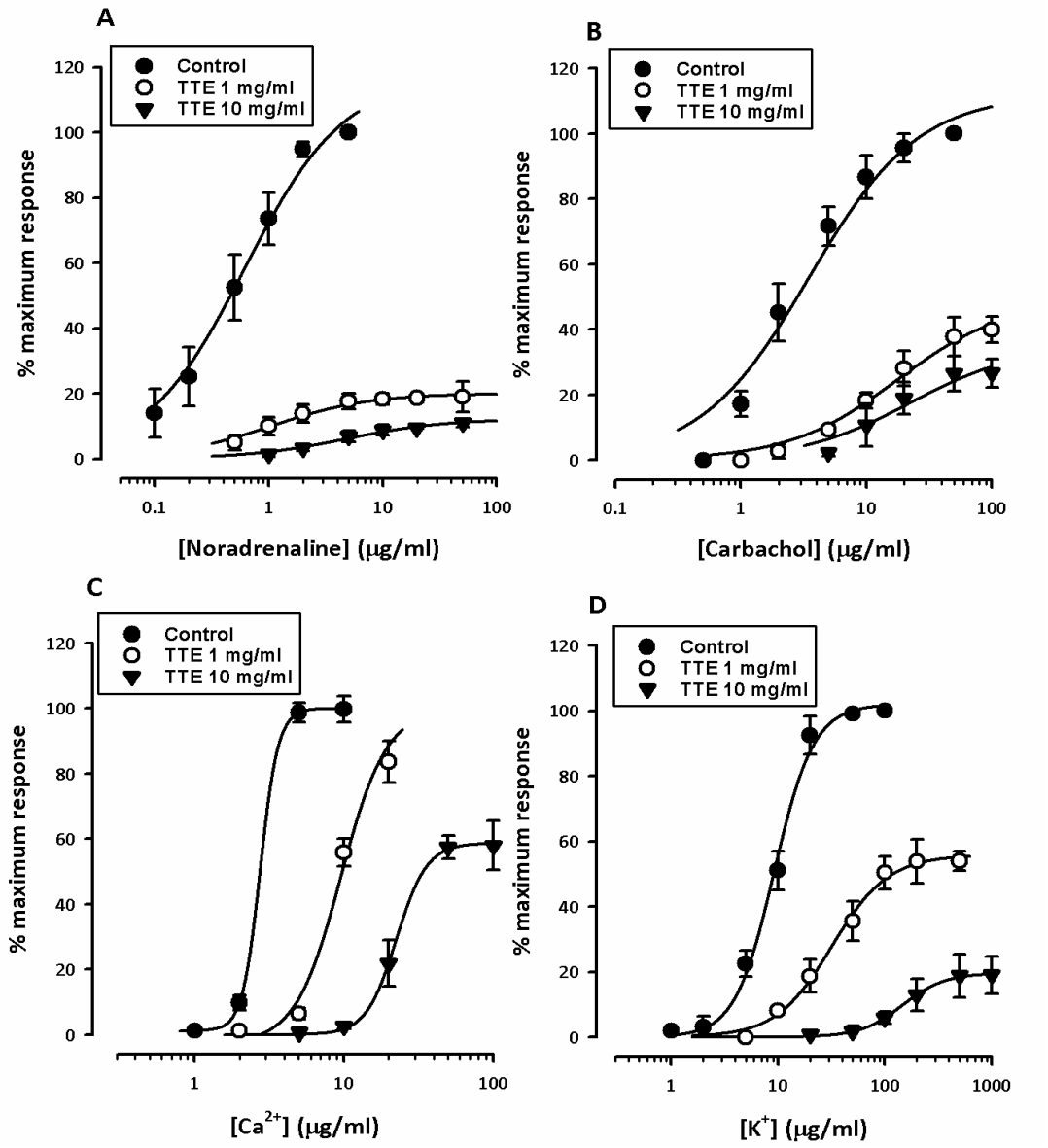

Fig. 3: **Effect of *T. tetraptera* on concentration-response curves of (a) noradrenaline, (b) carbachol, (c) calcium and (d) potassium on isolated rat anococcygeus preparation. Responses are expressed as percentages of maximum control response. Each point represents the mean ± S.E.M. (n = 3).**

Table 1: Emax, percentage inhibition, and EC50 of concentration-response curves for noradrenaline, carbachol, calcium and potassium in the presence of *T. tetraptera on isolated rat anococcygeus preparation (n = 3).*

	E_{max} (mm)	% Inhibition of E_{max}	EC_{50} (µg/ml)
Noradrenaline			
Control	42.67 ± 5.36	-	0.51 ± 0.04
TTE (1 mg/ml)	9.67 ± 1.45	81.94 ± 0.18	0.96 ± 0.01
TTE (10 mg/ml)	6.00 ± 0.57	89.82 ± 0.45	3.61 ± 0.04
Carbachol			
Control	44.67 ± 6.23	-	2.54 ± 0.03
TTE (1 mg/ml)	17.34 ± 0.33	58.13 ± 0.52	12.11 ± 0.01
TTE (10 mg/ml)	11.67 ± 0.67	74.86 ± 2.11	14.35 ± 0.70
Calcium			
Control	52.00 ± 1.36	-	2.70 ± 0.03
TTE (1 mg/ml)	48.67 ± 3.36	7.66 ± 1.28	7.18 ± 0.01
TTE (10 mg/ml)	30.00 ± 5.36	31.50 ± 2.20	27.29 ± 0.02
Potassium			
Control	49.33 ± 0.67	-	9.28 ± 0.02
TTE (1 mg/ml)	27.33 ± 9.84	41.08 ± 3.45	34.99 ± 0.05
TTE (10 mg/ml)	9.01 ± 5.57	81.59 ± 0.47	145.21 ± 0.02

that *T. tetraptera* is a non-specific antagonist at a number of receptor sites.

Isolated Rat Uterus Preparation

Having established the non-specific nature of extract on the anococcygeus muscle, we then investigated the nature of this interaction on isolated rat uterus. We chose the rat uterus because this plant is traditionally used in the prevention of post-partum contraction (Nwaiwu and Akah, 1986). Results obtained in the uterus were similar to that in the anococcygeus muscle. *T. tetraptera* (1-10 mg/ml) produced dose dependent relaxations (Fig. 1). The spontaneous movement of uterine smooth muscle is regulated by cycles of depolarization and repolarization. Action potentials appear at the height of depolarization and constitute a rapid influx of calcium via voltage-dependent calcium channels (Bolton, 1979).

Since *T. tetraptera* inhibits the spontaneous movements of the rat uterine smooth muscle, it may interfere with either the depolarization process or calcium influx through voltage-dependent calcium channels. Acetylcholine and potassium produce depolarization and contractions of uterine smooth muscle. The contraction is dependent on extracellular calcium that enters the cytoplasm either via the opening of voltage-dependent calcium channels or receptor-operated calcium-channels (Bolton, 1979). Atropine produced a parallel shift of the concentration-response curves to acetylcholine with similar maximum responses. *T. tetraptera* did not only produce a rightward shift of the concentration-response curves of both acetylcholine and potassium but also, it caused a depression of the maximum responses (Fig. 4 and 5). The EC_{50} and percentage inhibition values are as shown in Table 2.

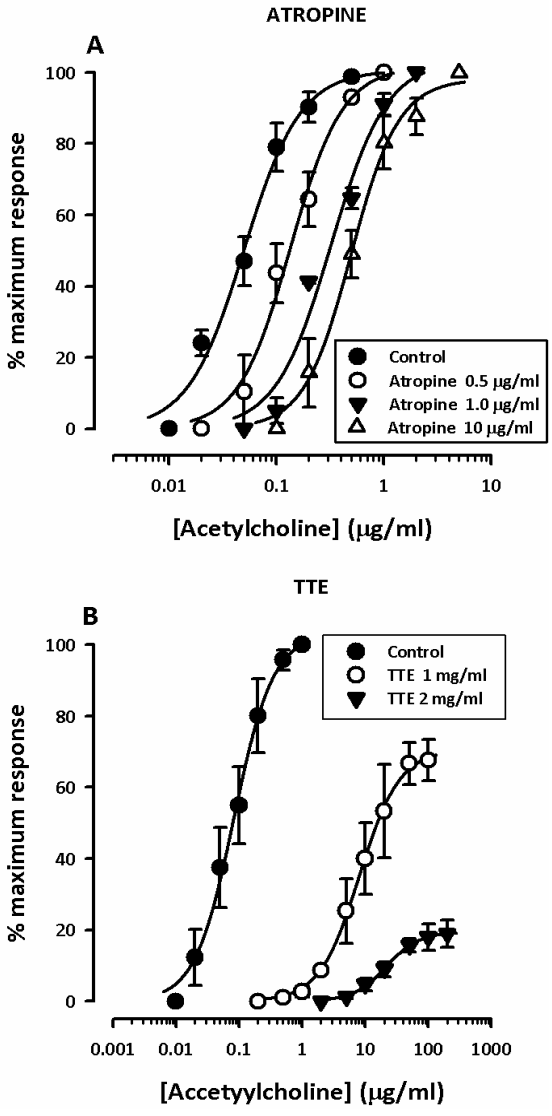

Fig. 4: Effect of (a) atropine and (b) *T. tetraptera* on concentration-responses curves of acetylcholine on isolated rat uterus preparation. Responses are expressed as percentages of maximum control response. Each point represents the mean ± S.E.M. (n = 3).

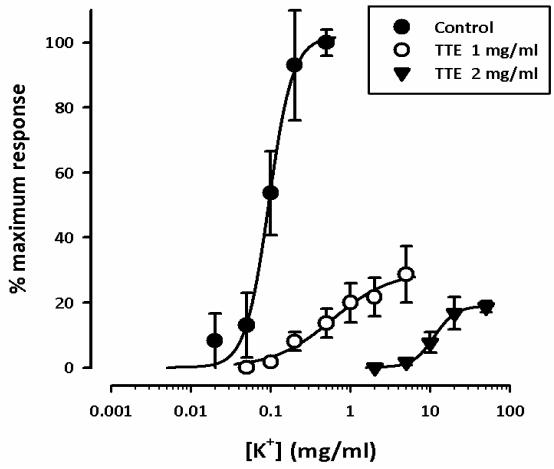

Fig. 5: Effect of *T. tetraptera* on concentration-responses curves of potassium on isolated rat uterus preparation. Responses are expressed as percentages of maximum control response. Each point represents the mean ± S.E.M. (n = 3).

Table 2: Emax and EC50 of concentration-response curves for acetylcholine and potassium in the presence of atropine, adrenaline and *T. tetraptera* on isolated rat uterus preparation (n = 3)

	E_{max} (mm)	EC_{50} (µg/ml)
Acetylcholine		
Control	50.67 ± 6.35	0.09 ± 0.13
Atropine (0.5 µg/ml)	50.00 ± 5.77	0.14 ± 0.07
Atropine (1 µg/ml)	50.66 ± 4.81	0.33 ± 0.06
Atropine (10 µg/ml)	49.83 ± 4.19	0.50 ± 0.06
Acetylcholine		
Control	57.67 ± 3.38	0.09 ± 0.18
TTE (1 mg/ml)	31.33 ± 8.67	6.67 ± 0.16
TTE (2 mg/ml)	8.67 ± 1.33	21.43 ± 0.17
Potassium		
Control	54.67 ± 2.91	0.08 ± 0.06
TTE (1 mg/ml)	26.67 ± 6.91	0.36 ± 0.25
TTE (2 mg/ml)	9.67 ± 0.33	11.14 ± 0.10

Isolated Guinea Pig Taenia Coli Preparation

After establishing the non-specific interaction of the extract with noradrenaline, acetylcholine, potassium and calcium, we set out to test our hypothesis. We hypothesized that since antagonism by *T. tetraptera* was non-specific, it may be acting on a site or sites in a common transduction pathway to prevent calcium influx, inhibit the calcium-induced calcium release mechanism, prevent the binding of calcium to calmodulin or through other unknown mechanism. To test this hypothesis, we tested the effect of the extract on taenia coli. Taenia coli is a smooth muscle widely employed in the studies of mechanisms involving calcium mobilization in excitation-contraction coupling as well as calcium antagonist properties of drugs (Nasu and Sasaki, 1998; Nasu, 1999). *T. tetraptera* (0.5-5 mg/ml) caused concentration-dependent relaxations of the guinea-pig taenia coli preparations (Fig. 1). It is not clear at this stage how *T. tetraptera* produces its inhibitory action. However, contraction of smooth muscle is dependent upon an increase in the concentration of cytoplasmic free calcium that activates the contractile elements, the source of which may be extracellular or intracellular (Bolton, 1979; van Breemen *et al.,* 1982). Therefore we explored the possibility that the inhibitory action of the extract might be through some calcium-regulated mechanism. Pre-treatment with *T. tetraptera* (1- 5 mg/ml) and nifedipine (0.01-0.1 µg/ml) for 15 minutes in Ca^{2+}-free high K^+ (100 mM) medium caused a shift to the right of the concentration-response curves for $CaCl_2$-induced contractions in high K^+ (100 mM) with depressed maximum responses (Fig. 6) suggestive of non competitive antagonism. The EC_{50} and percentage inhibition values are as shown in Table 3.

Isolated Guinea Pig Atria Preparation

For further studies on calcium antagonizing properties of the extract, we next used the isolated guinea–pig atria. Ca^{2+} antagonists are known to inhibit voltage-dependent calcium channels and to depress contractile force of vascular smooth muscle and cardiac muscle by decreasing transmembrane Ca^{2+} influx (Fleckenstein, 1977). Compounds that block L-type calcium channel on atria e.g. verapamil may cause decreased cardiac output and subsequently decrease the mean arterial blood pressure. These drugs are effective in the treatment of various cardiovascular disorders, chiefly angina pectoris, cerebral vasospasm and hypertension (Laragh *et al.,* 1987; Pepine *et al.,* 1983).

Table 3: EC_{50}, percentage inhibition and E_{max} values following 15 minutes pre-treatment with (a) nifedipine and (b) *T. tetraptera* on the cumulative concentration-response curves of $CaCl_2$–induced contractions of isolated guinea-pig taenia coli depolarized with K^+ (n = 3).

	E_{max} (mm)	% Inhibition of E_{max}	EC_{50} (µg/ml)
Nifedipine			
Control	52.00 ± 0.58	-	2.73 ± 1.35
Nifedipine (0.01 mg/ml)	47.67 ± 1.45	9.19 ± 2.50	3.78 ± 0.02
Nifedipine (0.1 mg/ml)	15.33 ± 1.20	72.05 ± 2.44	7.85 ± 0.12
TTE			
Control	51.33 ± 0.67	-	0.79 ± 0.02
TTE (1 mg/ml)	50.00 ± 0.58	1.00 ± 1.56	0.56 ± 0.07
TTE (2 mg/ml)	47.33 ± 0.88	9.73 ± 4.05	0.71 ± 0.05
TTE (5 mg/ml)	17.67 ± 1.76	63.75 ± 1.79	1.58 ± 0.06

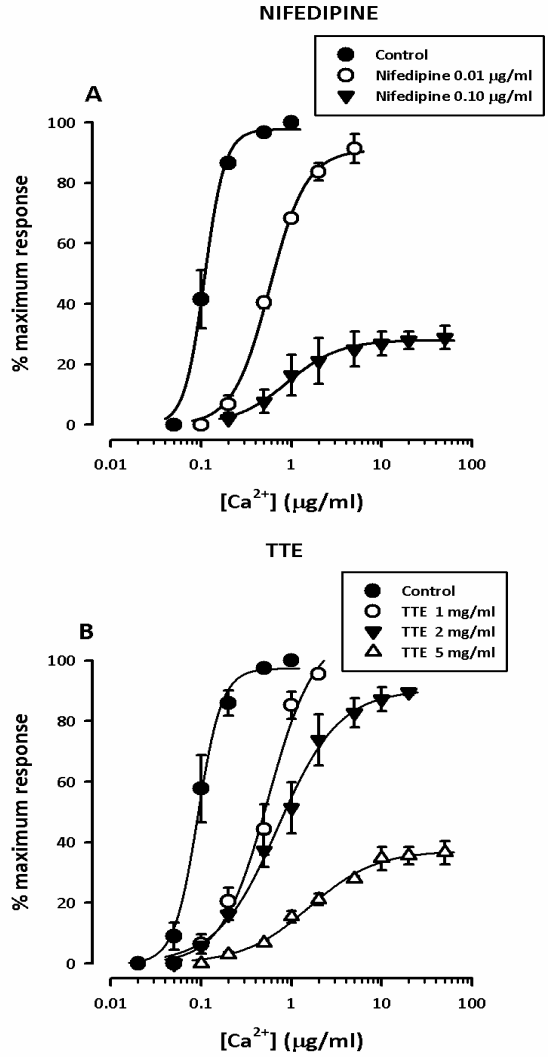

Fig. 6: Effect of (a) nifedipine and (b) *T. tetraptera* on concentration-response curves of $CaCl_2$-induced contractions of isolated guinea-pig taenia coli depolarized with K^+ (100 mM). The concentration-response relationships were obtained by addition of doses of $CaCl_2$ following 15 minutes pre-treatment with of nifedipine (0.01 and 0.1 µg/ml) in Ca^{2+} free, isotonic K^+ (100 mM) medium as described in the method. Responses are expressed as percentages of maximum $CaCl_2$-induced response. Each point represents the mean ± S.E.M. (n = 3).

Atrial muscle contraction of mammalian heart is dependent on extracellular calcium but is regulated from an intracellular compartment that stores calcium which can be released by the depolarization of the membrane. During the steady-state of the action potential, calcium flows from the extracellular space into the cell through L-type channels (Bean, 1985; Reiter, 1988). The effects of *T. tetraptera* and nifedipine on Ca^{2+} influx were examined on partially depolarised atria. These compounds produced not only a rightward displacement of the concentration-response curve for $CaCl_2$, but also a depression of the maximum response to $CaCl_2$ (Fig. 7). This suggests that the interaction between the compounds and calcium may not be competitive. The calcium inhibitory effect of *T. tetraptera* although several times weaker than that of nifedipine concerning potency, may thus contribute to the observed effects of the extract.

Drugs lower blood pressure by actions on peripheral resistance, cardiac output, or both. They can reduce peripheral resistance by acting on smooth muscle to cause relaxation of resistance vessels or by interfering with the activity of systems that produce constriction of resistance vessels (Hoffman, 2006). Calcium channel blockers such as nifedipine, which is widely used in Ghana for management of hypertension (Weber, 2002; Ohene Buabeng *et al*., 2004) produce their effect by binding the α_1-subunit of L-type calcium channel but to distinct sites, each of which interacts allosterically with each other and with the gating machinery of the channel, indirectly preventing diffusion of Ca^{2+} through its pore in the open channel (Katz, 1996). So far, the inhibitory effects of TTE observed were not only on receptor-mediated effects such as those produced by agonists e.g. noradrenaline and acetylcholine, but also on those produced by ions such as potassium and calcium. These observations coupled with the calcium inhibitory properties suggest that the effects of the extract are mediated partly by inhibition of calcium mobilization. Several compounds have been reported to block the contraction induced by

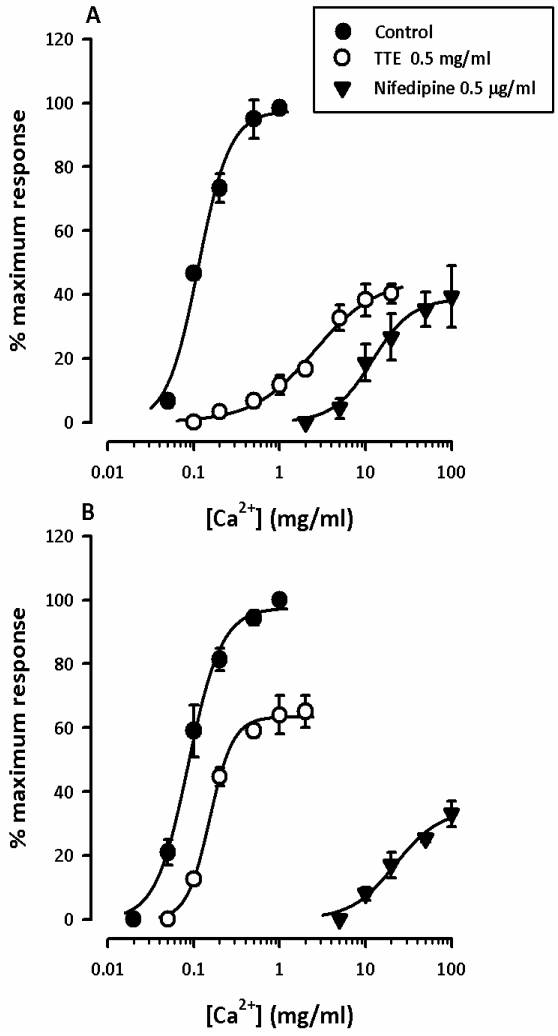

Fig. 7: Effect of *T. tetraptera (0.5 mg/ml) and nifedipine (0.5 µg/ml) on the concentration – response curves for $CaCl_2$-induced contractile force (A) and* rate of contraction (B) of isolated guinea-pig atria partially depolarized with K^+ (22 mM). The drugs were added 30 min before the cumulative addition of $CaCl_2$. Contractile forces and rates are expressed as percentages of the maximum contractile force and rate produced by $CaCl_2$. Each point represents the mean ± S.E.M. (n = 3).

potassium on several tissues (Godfraind and Kaba, 1971). Since these effects are overcomed by increasing the calcium concentration of the medium, it was proposed that it was due to the inhibition of the calcium function. These compounds include isoprenaline, local anaesthetics, anti-inflammatory drugs such as indomethacin, spasmolytics such as papaverine, sympatholytics such as dibenamine, antidepressants such as imipramine, neuroleptics such as chlorpromazine and vasodilators such as cinnarizine (Godfraind and Kaba, 1971). Since our extract blocked potassium induced contractions in the guinea-pig taenia coli and atria, we can thus conclude that it is acting similarly to the compounds listed above.

CONCLUSION

Results presented in this paper suggest that the extract may be acting further downstream of the signal transduction pathway, possibly at the level of calcium mobilization.

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