

## EFFECT OF ETHANOLIC EXTRACT FROM *ELAEOPHORBIA DRUPIFERA* LEAVES ON THE GASTROINTESTINAL SMOOTH MUSCLE OF THE RABBIT

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**Summary:** The crude extract from *E. drupifera* leaves was prepared using standard methods. The rabbit intestine was removed and separated into three segments (duodenum, jejunum and ileum). About 3-4cm of each segment was mounted in an organ bath containing Tyrode solution at  $37 \pm 1^\circ\text{C}$ . The spontaneous and rhythmic contractions were recorded and the effects of the crude extract (2-300 $\mu\text{g/ml}$ ) on the tissue responses were investigated. The effect of  $\text{Ca}^{2+}$  concentration and temperature of the bathing fluid were also studied. From the results, the extract (2-300 $\mu\text{g/ml}$ ) increased the amplitudes of contractions in a dose-dependent manner. However, regional differences occurred in the responsiveness of the tissue preparations. The  $\text{ED}_{50}$  values were found to be 25.12, 44.67 and 15.85  $\mu\text{g/ml}$  for the duodenum, jejunum and ileum respectively. Certain conditions such as calcium availability and increase in bath temperature favoured the action of the extract on the tissue preparations. Drugs like mepyramine or methysergide failed to influence the action of the extract. However, the extract-induced contractions were prevented or blocked by noradrenaline or atropine sulphate. The contractions were however ameliorated by the addition of acetylcholine or neostigmine to the bath solution. From the results, it is likely that the extract causes increased contractions of the tissue preparation via acetylcholine-like agent, which stimulates the muscarinic cholinceptors.

**Key Words:** *E. drupifera*, extract, leaves, intestinal contractions, increase, cholinergic.

### Introduction

Plants of the family Euphobiaceae are frequently used in indigenous practice of medicine. Their pharmacological properties include anti-tumor, antibacterial and anti hypertensive activities (Schiff; 1970). However, little literature is available on the medicinal uses of the species *Elaeophorbia drupifera* (Thonn.) Stapf, ("Akpa Mbief") although it is listed among the "plants that heal" (Ampofo, 1977). Ingenol (Kinghorn and Evans 1974; Abo, 1990) and lectins (Lynn and Radford, 1986) have been isolated from the latex of *E. drupifera*. The fruit is succulent (Kinghorn and Evans, 1974) but the latex has skin irritant effect (Kinghorn and Evans, 1975), and it is reported to promote inflammatory reactions (Abo, 1994). The leaf extract is said to contain hypoglycemic agent(s) (Eno and Itam, 1996) and stimulates autonomic cholinceptors in the rat uterus (Eno and Itam, 1997). Recently, the leaf

extract has been found to moderately inhibit HIV-1 and HIV-2 proviral and DNA copying (Ayisi and Nyadedzor, 2003).

This local herb is used by traditional herbalists for the treatment of hypertension, diabetes and many other ailments. Ground leaves (paste) are dissolved in either water or soft drink and administered orally in doses determined by age.

In the present study, our aim was to investigate the effects of the extract on the contractions of the small intestine, especially as the extract is administered orally. We investigated if there were regional differences in the smooth muscle responses to the extract, since each of the three segments of the small intestine is said to be anatomically different (Jimenez, *et al*, 1999; Kuriyama, *et al*; 1998; Koh, *et al*, 1998). We also tried to elucidate the possible mechanism of action employed by the crude extract.

## Materials and Methods

### (a) Preparation of the Crude Extract

Fresh leaves of *E. Drupifera* were collected, washed and freed of debris. The crude ethanolic extract was prepared according to the method described by Parry *et al.* (1987). Briefly, the wash water was blotted off and the leaves ground to paste using an electric grinder/blender. A quantity of ground sample (100g) was weighed and Soxhlet-extracted with 500ml absolute ethanol for 10hours at 100°C. The extract was then slowly evaporated to dryness in an electric oven at 40°C.

A starting sample of 100g of fresh material gave a mean yield of  $2.34 \pm 0.52\text{g}$  ( $\pm$  SD) of extract ( $n = 8$ ). Weighed samples of the extract were then used to make up test solutions of the desired concentrations.

### (b) The Effects of the Crude Extract on Intestinal Contractions

Rabbits of either sex (2-3kg) were killed by cervical dislocation. A midline incision was made at the abdomen to expose the small intestine. The three segments of the intestine were identified, separated, cut and dropped into beakers containing Tyrode solution of the following composition (mM concentrations): NaCl, 140; KCL, 2.7; NaHCO<sub>3</sub>, 12.0; MgCl<sub>2</sub>, 0.5; NaH<sub>2</sub>PO<sub>4</sub>, 0.3; CaCl<sub>2</sub>, 0.9 and glucose, 5.5. The solution was bubbled with air and maintained at  $37 \pm 1^\circ\text{C}$ . Short pieces (3-4cm) of each segment was cut and mounted vertically in a 25 ml organ bath containing Tyrode solution gassed with air. One end of a piece of tissue was tied to fixed support inside the organ bath, and the other end was connected to the polygraph (Grass Model 7D) via an isometric tension transducer (FT 0.03). A resting tension of 1g was maintained throughout the experiments. An equilibration period of about 30-min was allowed before the start of any experiment.

Various doses (2, 8, 32, 128, 300µg/ml) of the crude extract were added to the reservoir Tyrode solution bathing the tissue (duodenum, Jejunum or ileum), maintained at  $37 \pm 1^\circ\text{C}$  and allowed a contract time of about 5 min. to obtain a steady height of contraction. The amplitudes of contractions were measured in centimeters and them converted to grams (3cm deflection = 1g tension). The increase in twitch tensions (%) were then plotted against the log- concentration of extract.

### (c) Maintenance of Extract-induced Increase of Ileum Response

The ileal preparation was mounted in an organ bath and bathed with a reservoir Tyrode

solution as described in section (a) of the Methods section.

After 30min. equilibration period, the crude extract (10µg/ml) was applied at zero time. In the continued presence of the extract, the amplitude of contraction was recorded at 30min interval for 3hrs without wash ( $n = 5$ ). In another group of ileal preparations, the same procedure was employed but the preparations were repeatedly washed ( $n = 5$ ) with Tyrode solution at 30min. interval for 3hours. A total volume of 300ml of Tyrode solution was used for the 3hrs. duration.

### Temperature Dependency

Since the rate of many biochemical reactions are temperature dependent, it was necessary to study the effect of temperature on extract-induced increase in the contractions of the duodenum, jejunum and ileum. The tissue preparations were treated with the extract (10µg/ml) from *E. drupifera* leaves at various temperatures (20, 25, 30, 35, 40 °C) of the reservoir Tyrode solution containing low calcium (0.5 mM). The mechanical responses of the tissue preparations ( $n = 5$ ) at the different temperatures were recorded, and the increases in responses (% control) were plotted against their corresponding temperatures

### (e) Effect of Calcium Concentration

To specify the conditions under which the increase in twitch tension by the extract became apparent, we first examined the effects of decreasing reservoir calcium concentrations. Tyrode solutions containing low calcium concentrations (0.1, 0.3, 0.5, 0.7, 0.9mM) were prepared and used as the bathing solutions for the isolated tissues (duodenum, jejunum, ileum) experiments ( $n = 5$ ). The crude extract (10µg/ml) was tested on each intestinal segment at the low Ca<sup>2+</sup> medium.

Effect of some Pharmacological agents on Extract-induced Contractions of the Rabbit Ileum

The extract. (10µg/ml)-induced increase in the contractile response of the ileum was challenged with different pharmacological agents. The agents were mepyramine ( $2.8 \times 10^{-6}\text{M}$ ), methysergide ( $1.13 \times 10^{-5}\text{M}$ ), neostigmine ( $1.64 \times 10^{-6}\text{M}$ ), potassium chloride (50 mM), atropine sulphate ( $2.9 \times 10^{-6}\text{M}$ ), acetylcholine ( $1.1 \times 10^{-7}\text{M}$ ) and noradrenaline ( $1 \times 10^{-7}\text{M}$ ).

### Statistical Analysis

Regression lines with confidence limits were calculated for the linear portions of log-concentration response curves. The log-concentration limits at 50% of the maximum response were used in the analysis of the

significance of concentration differences as described by Birmingham et al, (1970). Maximum responses were compared by unpaired student's t-test.

### Drugs

Atropine sulphate, noradrenaline, and acetylcholine chloride were from Sigma (U.S.A.). Methysergide from Sandoz, Brazil. Mepyramine and neostigmine from Roche, Brazil

### Results

#### (a) Effect of Extract on Intestinal Contractions

The rabbit intestine (*Duodenum*, *Jejunum* and *Ileum*) showed spontaneous and rhythmic contractions in Tyrode solution. Although the amplitudes of these contraction varied from one preparation to another, the mean amplitudes (*twitch heights*) converted to gram tension were about  $1.5 \pm 0.7$ g,  $1.2 \pm 0.5$ g, and  $1.8 \pm 0.4$ g, for the duodenum, jejunum and ileum respectively. The latencies of responses were about 2-3 sec. in all segments.

The results show that the crude extract from *E. Drupifera* leaves (2-300  $\mu$ g/ml) dose-dependently increased the amplitudes of contractions in the duodenum, jejunum and ileum with ED<sub>50</sub> values of 25.12, 44.67 and 15.85  $\mu$ g/ml respectively (Fig.1). High doses of the extract (above the ED<sub>50</sub> values) produced dose-dependent sustained contractions which were characterized by a rise in basal tone. Based on these ED<sub>50</sub> values, the test dose of 10  $\mu$ g/ml was selected. The dose-response relationships (Fig.1) was apparently sigmoidal, and for each curve, a straight line regression was fitted for the linear portion of the curve, from which the ED<sub>50</sub> values were determined.

Figure 1, shows regional difference in the responsiveness of the intestinal smooth muscle to various doses of the extract. From Fig.1, it is clear that the ileum is the most responsive segment to any given dose of the extract while the jejunum is the least responsive. For instance, the maximum dose tested (300  $\mu$ g/ml) produced about 83.8%, 68.9%, and 88.7% increase in responses of the duodenum, jejunum and ileum respectively.

#### (b) Maintenance of Extract-Induced Increase of Ileum Response

The effects of prolonged exposure of the tissue preparation (*Ileum*) to the extract was also investigated (Fig 2). The crude extract (10  $\mu$ g/ml) was applied to the ileal preparation at zero time. In the continued presence of the

extract for 3hrs (n = 5), the increase in contraction was maintained by about  $86.5 \pm 3.2\%$  without wash. When the preparation was repeatedly washed (n = 5) with Tyrode solution (300ml) at 30 min interval, there was partial recovery as the twitch heights decreased towards control levels time-dependently. However, at the end of the exposure period (3hr), about  $55 \pm 4.1\%$  increase in contraction was still maintained (Fig.2).

#### (c) Temperature Dependency

The tissue preparations were treated with the extract (10  $\mu$ g/ml) from *E. Drupifera* leaves at various temperatures (20, 25, 30, 35, 40 °C) in Tyrode solution containing low calcium (0.5 mM Ca<sup>2+</sup>). The results (Fig. 3) showed that in all three segments of the small intestine, the extract (10  $\mu$ g/ml) in low extracellular Ca<sup>2+</sup>, caused increased responses which were temperature-dependent. At the lowest temperature (20 °C), the extract produced about  $18.8\% \pm 5.1$ ,  $17.9\% \pm 5.5$  and  $19.4\% \pm 3.2$  increases in responses of the duodenum, jejunum and ileum respectively. The highest temperature tested (40 °C) caused, about  $64.6\% \pm 4.7$ ,  $59.8\% \pm 4.4$  and  $71.4\% \pm 3.9$  increases in the responses of the duodenum, jejunum and ileum respectively. The results showed that the ileum, followed by the duodenum, was more responsive than other segment. (Fig.3).

#### (d) Effect of Calcium Concentration

The increase in twitch tension by the extract was apparent when the calcium concentration in the Tyrode solution was 0.5mM. The results is summarized in Table 1. At 0.5 mM calcium, the amplitudes of the twitch tensions of the extract-untreated muscles were 48.7%, 54.1% and 42.2% (for the duodenum, jejunum and ileum respectively) of those at 0.9 mM calcium, and the increase of the twitch tension by the extract became significant. The degree of the increase of the twitch tension decreased as the concentration of Ca<sup>2+</sup> in Tyrode solution decreased to 0.1mM calcium. The increase in twitch tension induced by the extract (10  $\mu$ g/ml) reached 73.3%, 66.7% and 78.1% in the duodenum, jejunum and ileum respectively. The correlation coefficient between degrees of increase of twitch tension and decrease in reservoir Ca<sup>2+</sup> concentrations was calculated as -0.97. The absolute value of the tension before the treatment in the Tyrode solution containing 0.1mM Ca<sup>2+</sup> was as low as  $0.38 \pm 0.14$  g (mean  $\pm$  S.D; n = 5). (Table1).

Table 1. Effect of Reservoir  $Ca^{2+}$  Concentration on Extract-Induced Increase in Twitch Tension.

Ca <sup>2+</sup> Concentration (mM)	No. of Expts.	% decrease by medium change			% increase by the extract (10µg/ml)		
		Duodenum	Jejunum	Ileum	Duodenum	Jejunum	Ileum
0.9	5	-	-	-	73.3 ±9.9	66.7±13.7	78.1 ±7.2
0.7	5	18.4 ±5.8	21.8 ±10.6	11.1 ±9.2	64.7 ±7.4	54.8 ±7.7	72.5 ±12.1
0.5	5	48.7 ±7.1	54.1 ±13.3	42.2±9.6	47.2 ±9.2	42.3±11.1	53.5 ±8.3
0.3	5	80.4 ±13.2	87.4 ±9.1	78.1±11.5	17.9 ±6.4	14.2 ±5.3	21.8 ±6.8
0.1	5	87.8 ±10.7	92.3 ±11.6	82.4±4.3	12.3 ±4.9	10.7 ±6.8	14.5 ±5.2

Each value represents mean ± S.D. n = 5

\*Percent decrease of twitch tension caused by lowering  $Ca^{2+}$  concentration in medium from 0.9 mM to the concentration indicated. Values were calculated as follows:-

$$\text{value} = \frac{(\text{tension at } 0.9 \text{ mM } Ca^{2+}) - (\text{tension at indicated } Ca^{2+})}{(\text{tension at } 0.9 \text{ mM } Ca^{2+})} \times 100$$

\*\*Percent increase of twitch tension at indicated  $Ca^{2+}$  concentration by treatment with the extract (10 µg/ml). Values were calculated as follows:-

$$\text{value} = \frac{(\text{tension before extract-treatment}) - (\text{tension after extract-treatment})}{(\text{tension before extract-treatment})} \times 100$$

Effects of some pharmacological agents on Extract-induced contraction of the rabbit ileum.

Table 2 summarizes the effects of various pharmacological agents on the contractions induced by the crude extract (10 µg/ml). The crude extract (10 µg/ml) increased the contraction of the ileum by about 28.4 ± 3.8 % (SEM, n = 5) (Fig.4a). Mepyramine ( $8 \times 10^{-6}M$ ) and methysergide ( $1.13 \times 10^{-5}M$ ) failed to prevent or block the extract-induced contractions (Fig. 4b-c). However, both neostigmine ( $1.64 \times 10^{-6}M$ ) and KCl (50mM) potentiated the extract-induced contractions (Figs. 4d and 5a). Atropine sulphate ( $2.9 \times 10^{-6}M$ ) and Noradrenaline ( $1 \times 10^{-7}M$ ) both blocked or abolished the contractions (Fig.5b & d) while acetylcholine ( $1.1 \times 10^{-7}M$ ) enhanced the contraction (Fig. 5c).

Table 2: The effect of some pharmacological agents on extract-induced contraction of the rabbit ileum.

Tissue Tension (g)		Drug-induced Tension		% Change in Response [Ext. Vs Drugs]
Control	Extract-treated (10µg/ml)	Drugs	Tension Produced (g)	
1.12 ± 0.05	1.43 ± 0.35	-	-	-
1.22 ± 0.03	1.45 ± 0.06	Mepyramine ( $2.8 \times 10^{-6}$ )	1.45 ± 0.25	0
1.16 ± 0.23	1.38 ± 0.08	Methysergide ( $1.13 \times 10^{-5}M$ )	1.38 ± 0.07	0
1.08 ± 0.31	1.32 ± 0.15	Neostigmine ( $1.64 \times 10^{-6}M$ )	1.96 ± 0.04	48.5% Increase
1.18 ± 0.34	1.34 ± 0.41	Potassium Chloride (50mM)	1.88 ± 0.12	40.2% Increase
1.20 ± 0.42	1.46 ± 0.32	Atropine Sulphate ( $2.9 \times 10^{-6}$ )	0.13 ± 0.02	89.7% Decrease
1.02 ± 0.08	1.31 ± 0.09	Acetylcholine ( $1.1 \times 10^{-7}M$ )	1.95 ± 0.06	48.9% Increase
1.23 ± 0.05	1.44 ± 0.25	Noradrenaline ( $1 \times 10^{-7}M$ )	0.56 ± 0.04	61.2% Decrease

The effect of mepyramine, methysergide, neostigmine, potassium chloride, atropine, acetylcholine and noradrenaline on the amplitude of contraction of the rabbit ileum (converted to gram tension) induced by the crude extract (10µg/ml). Data represents mean values ± S.E.M. (n = 5).

Fig.1 Dose-effect relationship. The effect of administering graded doses (2-300 $\mu$ g/ml) of the crude extract to the tissue preparations (Duodenum  $\bullet$ ); Tejunm  $\blacksquare$ ); and Ileum ( $\blacktriangle$ ) Data represents mean value  $\pm$  SD, n=5.

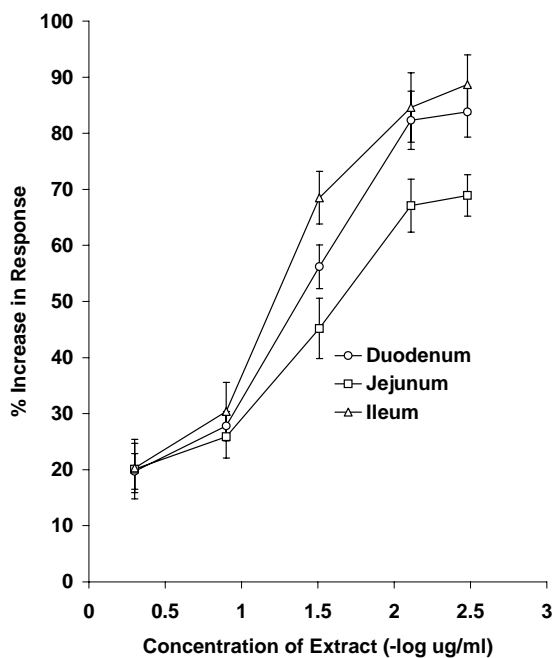


Fig.2 Maintenance of extract-induced contraction of the isolated rabbit ileum, and the effect of washing the tissue preparation at 30min. interval. Given are the mean values  $\pm$  SD.

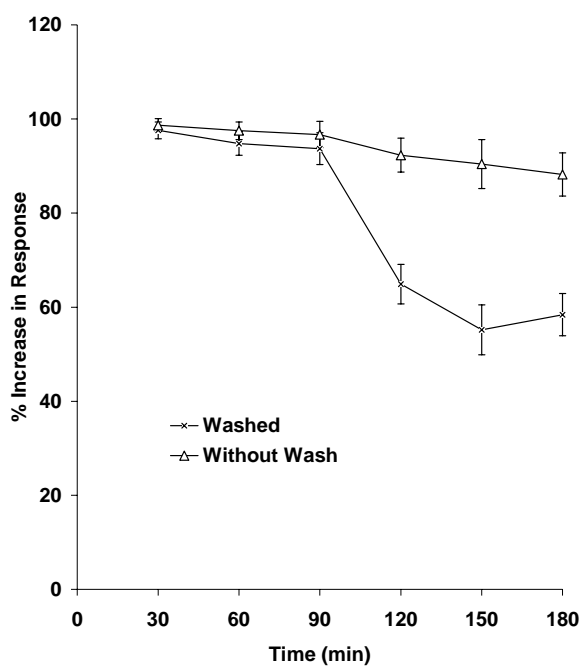


fig.2

Fig.3 Effect of bath temperature on the extract-induced increase in twitch tension of the isolated rabbit duodenum ( $\Delta$ ); jejunum ( $\bullet$ ); and ileum ( $\square$ ). Results are shown as means  $\pm$  SD, n = 5.

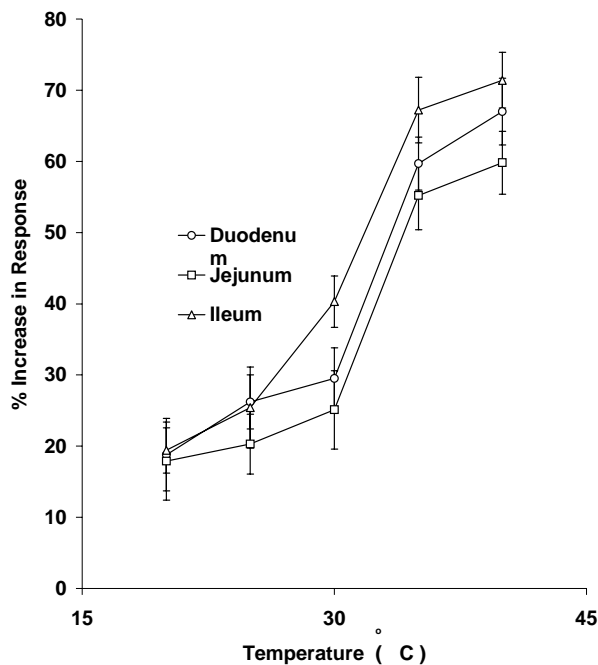


fig.3

Fig.4 Typical mechanical recordings showing the effects of mepyramine (Mepy), methysergide (Methy), and neostigmine (Neo) on extract-induced contraction of the ileum.

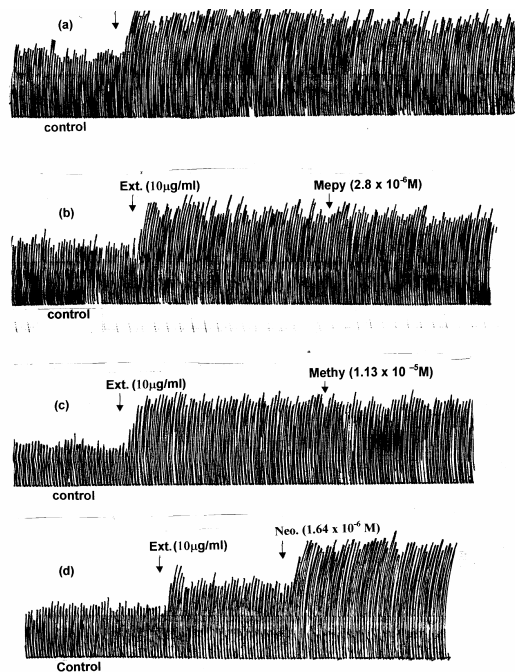
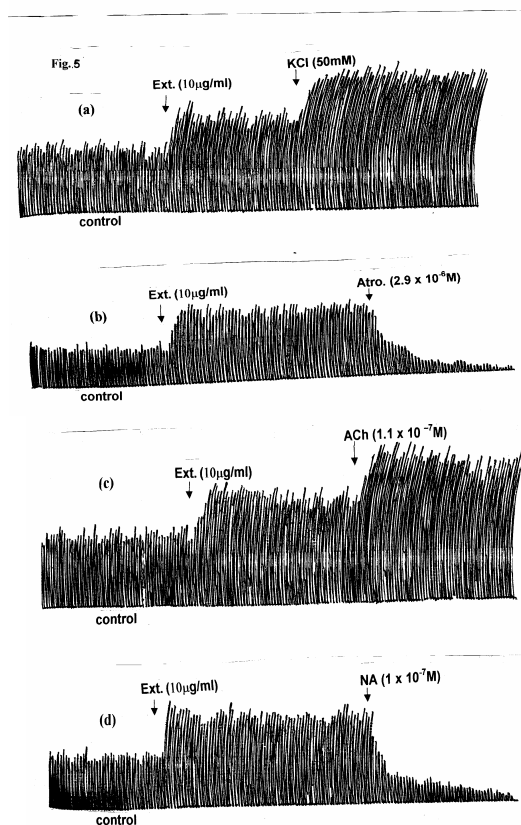


Fig.5 Typical mechanical recordings showing the effects of potassium chloride (KCL), atropine sulphate (Atr.), acetylcholine (Ach) and noradrenaline (NA) on the extract-induced contraction of the rabbit ileum.



### Discussion

Earlier studies have shown a low toxicity of *E. drupifera* leaf extract. The high LD<sub>50</sub> value (135.6 mg/kg. mine, i.p) shows its low acute toxicity (Eno, *et al* 1999). From the current study, it is evident that this extract contains agent(s) capable of stimulating the intestinal smooth muscle in a dose-dependent manner. Regional differences exist in the responsiveness of the intestinal muscles (duodenum, jejunum and ileum), to the various doses of the extract (Kuriyama, *et al*, 1998; Koh, *et al*, 1998 Jimenz, *et al*, 1999; Huang, *et al*, 1999). It is very unlikely that these differences were caused by agents in the crude extract since the differences existed even without the application of the extract. Therefore, anatomic differences in the three segments of the small intestine could be a more likely explanation. With or without the extract, the ileum was the most responsive

while the jejunum was the least, in the present study. The intestinal smooth muscles have spontaneous myogenic activities which are generated by pacemaker potentials or slow depolarization of the membrane (slow waves), although these waves may be markedly influenced by nervous activity (Kuriyama, *et al*, 1998 Koh, *et al*, 1998,). It is the slow fluctuations (waves) that modulate the spike activity and spike frequency (Koh, *et al*, 1998,). Therefore, the extract-induced increase in amplitude of the responses in all three segments of the intestine was probably due to, the enhancement of the slow wave activity by the extract. Slow waves can normally be recorded in both circular and longitudinal muscles of the small intestine (Ward *et al*; 2000). They are produced by specialized pacemaker cells located in the circular and longitudinal muscle layers of the intestine (Torihashi, *et al*, 2002). The sites of

origin of the slow waves, their method of propagation and the interaction between the circular and longitudinal layer are the factors responsible for the regional differences in the responsiveness of the tissue preparation (Jimenez, *et al*, 1999). For instance, in the rabbit jejunum, the slow wave is smaller in the longitudinal muscle than in the circular muscle whereas in the ileum, the wave is large in both muscles (Huang *et al*, 1999). Therefore, the regional differences in the responsiveness of the tissue preparations, is not surprising.

The enhancement of the slow wave activity (spontaneous contractions or slow fluctuations) by the extract, with the resultant increase in spikes (contractions) is also not surprising. This is because, as shown in Table 1 the extract-induced increase in contraction is dependent on the reservoir calcium concentration. The higher the extracellular calcium concentration, the greater the extract-induced increase in contraction. Slow depolarization of the membrane opens voltage-dependent calcium channels, and calcium ions enter the cell to induce the release of calcium from the sarcoplasmic reticulum (ie; the calcium-induced calcium then binds to calmodulin to form  $Ca^{2+}$ - calmodulin complex. It is this complex that triggers other chains of events that leads to smooth muscle contraction (Bolton, 1979). Therefore, calcium availability is a necessary condition for the action of the extract. It is probable that agent(s) in the extract cause improved cytosolic calcium. However, even in low calcium concentration (0.5mM), increase in reservoir temperature also increased the activity of the extract on the tissue preparation. This temperature dependency could be due to increased rate of biochemical reactions at higher temperatures (Guyton and Hall 2000). Interestingly, the extract-induced contraction could be maintained for about 3 hr. without wash, and even when washed at 30min. interval, the recovery was never complete (Fig. 2). This unique property of the extract is important since the duration of action of a drug is of high clinical value. (Bowman and Rand, 1980).

Attempts were made to elucidate the possible mechanism of action employed by the extract (Table 2). The failure to influence the action of the extract with mepyramine and methysergide (Fig. 4a) (Fig. 4b-c) suggests that the extract is not utilizing histamine and 5-HT pathways respectively. The potentiation of extract-induced responses by potassium chloride was probably via a non-neural mechanism. Potassium ions directly depolarize the cell membrane, and as such no

receptors are involved (Bolton, 1979, Parry, *et al*, 1996, Parry and Duri, 1994). However, that atropine sulphate prevented or abolished extract-induced contraction strongly suggests a cholinergic mechanism for the action of the extract.

That neostigmine, an anticholinesterase which facilitates cholinergic transmission and acetylcholine, both ameliorated the action of the extract, are all in line with this contention. However, noradrenaline (NA) reversed the action of the extract, possibly by a direct effect on the tissue.

In conclusion, regional differences exist in the responsiveness of the small intestine. The differences could be purely anatomic such as distribution of specialized pacemaker cells in the circular and longitudinal layers. In all segments of the tissue, the extract caused increased contractions, probably by increasing the cytosolic calcium concentration. Finally, the extract probably contains an acetylcholine-like agent that is capable of stimulating the muscarinic cholinergic receptors.

However, it is premature to speculate on the actions of this extract on smooth muscle since it may contain more than one active compound in its crude stage. Further progress must await refinements in its separation techniques.

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