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Ethnopharmacological activities of the leaves of *Cycas circinalis* (Cycadaceae)

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ABSTRACT

The leaves of the plant *Cycas circinalis* (Cycadaceae) are usually prescribed with other part of the plant traditionally as a purgative the pharmacological activities of the leaves as a purgative was evaluated on isolated rabbit and guinea pig ileum against two spasmogen, Acetylcholine (Ach), and Histamine (Hist). The aqueous solution of fractions 'AC', 'EA,' 'CC' abolished completely Acetylcholine and Histamine induced contraction (dose dependant), the effect of the extract-mimicked antagonists, (atropine, Atr, and adrenaline, Adr,). Since atropine and other cholinergic antagonist blocks the colic and spastic action brought about by Ach on intestinal muscle, they are therefore beneficial in colic spasm and increase in parastalsis. Fractions 'AC', 'EA' and 'CC' of *Cycas circinalis* leaves also antagonize Ach induced contraction on isolated rabbit and guinea pig ileum so they have anticholinergic activities. Since anticholinergy cannot be as purgative hence the pharmacological action shows the leave could then be a synergistic part (synergism). This been the first report on pharmacological action on *Cycas circinalis* leaves

Keywords: Ethnopharmacology; *Cycas circinalis;* Cycadaceae

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INTRODUCTION

The plant *Cycas circinalis* (Cycadaceae) is a gymnosperm [1], a small monocaulous savanna tree reaching a height of 5 meters [5]. Cultivated in southern Africa, China, and Japan, Sparingly cultivated in India and Nigeria also found in eastern tropical Africa [1]. They are use traditional as food, decoction, and reclamation of moving sand. Report on the family of the plant shows that a species is use as a purgative by the Zulu's [14]. In Papua-New Guinea the dried leaf of *Cycas circinalis* is use in the screening of anti bacterial and anti tumor activities [13]. Fresh leaf in India when screened for anti fungal activities is active [11]. The fruit unripe is used as food in Papua New Guinea, adult takes it orally as decoction and it is also used for treating wounds [6]. For carcinogenic activity, dried tuber of Nigeria species when administered on mouse at a dose of 5.0% diet is active, associated with an increase of colon cancer. A high fibre diet showed protection against development of colon cancer [3].

Species of strangeria is much used by the Zulus as medicine, the tuberous root for a wide variety of conditions and the underground stem as a purgative. The seed is also purgative and some parts of the plant are apparently edible [14].

Extraction

Dried and coarsely powdered leaves of *Cycas circinalis* (120g) were refluxed with petroleum ether (60-80^{o}C) for 10 hours. The extract was decanted off and fresh quantity of the petroleum ether was added again and refluxed for another 10 hours. The defatted leaves were completely dried and extracted with acetone. The combine acetone extracts where concentrated on water bath whereby a highly viscous greenish – brown mass was obtained. This was refluxed with petroleum ether (60-80^{o}C) and benzene successively until the solvent in each case was almost colourless. The residue left behind was then treated with hot water. The water insoluble portion was dissolved in acetone and dried under reduced pressure. A solid brown residue (8.6g) obtained responds to usual flavonoidal colour tests were marked 'AC' The aqueous solution was extracted with ethyl acetates successively. The ethyl acetate extracts where combined and the solvent was recovered under reduced pressure. The semi- solid residue thus obtained was marked 'EA' and respond to usual flavonoidal test. The residue left behind was extracted with chloroform successively. The Chloroform extracts where combined and the solvent was recovered under reduced pressure. The semi- solid residue thus obtained was marked 'CC' and respond to usual flavonoidal test

Experimental procedure

An unknown weight of *Cycas circinalis* leaves was collected around Tashar Fulani in Sakaru village, Jos road Zaria in June of 2002, identified by A.B.U. Herbarium and was dried and grounded to powder. 120g of the powdered leaves were extracted to exhaustion using acetone, ethyl acetate and chloroform successively by cold process (maceration) [8].

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MATERIALS AND METHOD

INSTRUMENT

Recording micro dynamometer Ugo-Basile model No.7050 for recording isometric response of various isolated tissues, aerating gas (oxygen), petri dish and thermo circulator.

ANIMALS

The animals' guinea pigs 352-486g and rabbits 1.28kg –1.63kg were purchased and bread in the animal house of the faculty of pharmaceutical science A.B.U. Zaria. The animals where maintain with local animal feed. They were handled humanely according to the international ethical committee on animal handling.

REFERENCE DRUGS AND SAMPLES

The reference drugs and samples were prepared in aqueous solution; they include Acetylcholine (Ach), atropine (atr), Histamine (Hist), adrenaline (Adr), (this were obtained from sigma and BDH chem. Co.) aqueous solution of fractions 'CC', 'EA', 'AC'.

EXPERIMENTAL METHOD

The physiological solutions [12] were freshly prepared on the day of the experiment. The reference drugs were prepared by dissolving directly the quantity required in normal saline or (distilled water). E.g. for acetylcholine solution (Ach) a stock of $10^{-1}g/dm_3$ = 0.55m keep at pH4 which is stable, was made by dissolving 10g of Ach in 100ml of de-ionized water, subsequent dilutions were made to the required strength of each drug, except for adrenaline with which equal amount of sodium metabisulphate was added as stabilizer.

ISOLATED TISSUE PREPARATION

The animals used (rabbit and guinea pigs) were staved for one day before used and were killed by a blow on the head.

The blood was drained through an incision in the throat. The abdomen opened and pieces of ileum were isolated and placed in a petri dish containing the physiological solution aerated by oxygen pump. The tissue were transferred to an organ bath and allowed to stabilized for about 2hours before studying the effect of the drugs on them and the temperature was set thermostatically at 37% [10].

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RESULT

TABLE 1 Effect of 5μg/Ml Acetylcholine (Ach) on isolated rabbit ileum

Vol. Injected	Organ bath Conc. μg/Ml)	Conc. In ng/Ml	Contraction Response Mm	Log dose	%response
0.05	0.01	10	4.0	1	49
0.1	0.02	20	6.3	1.3	77
0.2	0.04	40	8.2	1.6	100

TABLE 2 Effect of 100 μg/Ml Histamine on isolated rabbit ileum

Vol. Injected	Organ Bath Conc. μg/Ml	Conc. In ng/ml	Contraction response Cm	Log dose	%response
0.1	0.4	400	2.6	2.6	47
0.2	0.8	800	3.4	2.9	52
0.4	1.6	1600	4.6	3.2	84
0.8	3.2	3200	5.5	3.5	100

TABLE 3 Effect of 5μ/Ml Atropine on isolated rabbit ileum

Vol. Injected	Organ Bath Conc. μg/Ml	Conc. In ng/Ml	Relaxation response	Log dose	%response
0.05	0.01	10	12	1.0	29
0.1	0.02	20	2.8	1.3	67
0.2	0.04	40	4.2	4.2	100

TABLE 4 Effect of 5μg/Ml Adrenaline on isolated rabbit ileum

Vol. Injected	Organ Bath Conc. μg/Ml	Conc. In ng/Ml	Relaxation response	Log dose	%response
0.05	0.01	10	3.1	1.0	79
0.1	0.02	20	3.5	1.3	90
0.2	0.04	40	3.7	1.6	95
0.4	0.08	80	3.9	1.9	100

TABLE 5 Effect of 10Mg/Ml of fraction 'AC' on isolated rabbit ileum

Vol. injected	Organ Bath Conc. Mg/Ml	Conc. In ng/Ml $x10^3$	Relaxation response	Log dose	%response
0.05	0.02	20	0.6	4.3	55
0.1	0.04	40	0.8	4.6	73
0.2	0.08	80	1.1	4.9	100
0.4	0.16	160	0.8	5.2	73

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TABLE 6 Effect of 10Mg/Ml of fraction 'CC' on isolated rabbit ileum

Vol. injected	Organ Bath Conc. Mg/Ml	Conc. In ng/Ml x10^3	Relaxation response	Log dose	%response
0.05	0.02	20	1.9	4.3	70
0.1	0.04	40	2.2	4.6	81
0.2	0.08	80	2.7	4.9	100
0.4	0.16	160	2.0	5.2	74

TABLE 7 Effect of 10Mg/Ml fraction 'EA' on isolated rabbit ileum

Vol. injected	Organ Bath Conc. Mg/Ml	Conc. In ng/Ml x10^3	Relaxation response	Log dose	%response
0.1	0.04	40	-	4.6	0
0.2	0.08	80	1.3	4.9	65
0.4	0.16	160	1.5	4.5	75
0.8	0.32	320	2.0	5.5	100

TABLE 8 Effect of varying dose of 5µg/Ml Ach against 0.1Ml of 5µg/Ml atropine

Vol. Injected	Organ Bath Conc. µg/Ml	Conc. In ng/ml x10^3	Inhibition response	Log dose	%response
0.1	0.02	20	-	1.3	-
0.2	0.04	40	2.0	1.6	24
0.4	0.08	80	3.5	1.9	43

TABLE 9 Effect of varying dose of 10Mg/Ml of fraction 'CC' against 0.2ml of 5µg/Ml Ach

Vol. injected	Organ Bath Conc. Mg/Ml	Conc. In ng/Ml x10^3	Inhibition response	Log dose	%response
0.1	0.04	40	8.0	4.6	98
0.2	0.08	80	6.4	4.9	78
0.4	0.16	160	5.2	5.2	63
0.8	0.32	320	4.2	5.5	51
1.6	0.64	640	3.0	5.8	37

TABLE 10 Effect of varying dose of 10Mg/Ml of fraction 'EA' against 0.2ml of 5µg/Ml Ach

Vol. Injected	Organ Bath Conc. Mg/Ml	Conc. In ng/Ml x10^3	Inhibition response	Log dose	%response
0.1	0.04	4.0	5.3	4.6	65
0.2	0.08	80	4.9	4.9	60
0.4	0.16	160	4.7	5.2	57
0.8	0.32	320	2.9	5.5	35
1.6	0.64	640	-	5.8	-

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TABLE 11 Effect of varying dose of 10Mg/Ml of fraction 'CC' against 0.2ml of 100µg/Ml Histamine

Vol. Injected	Organ Bath Conc. Mg/Ml	Conc. In ng/Ml x10^3	Inhibition response	Log dose	%response
0.1	0.04	4.0	1.2	4.6	22
0.2	0.08	8.0	0.7	4.9	13
0.4	0.16	160	0.6	5.2	11
0.8	0.32	320	-	5.5	-

TABLE 12 Effect of varying dose of 10Mg/Ml fraction 'EA' against 0.2ml of 100 µg/Ml Histamine

Vol. Injected	Organ Bath Conc. Mg/Ml	Conc. In ng/Ml x10^3	Inhibition response	Log dose	%response
0.1	0.04	4.0	0.6	4.6	11
0.2	0.08	80	0.4	4.9	7
0.4	0.16	160	-	5.2	-

Chart 1: Effect of 5ug/ml acetylcholine on rabbit ileum

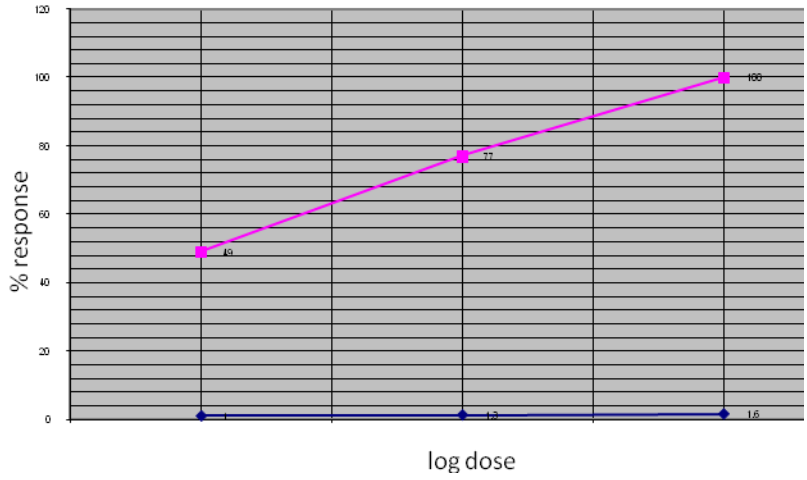

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Chart 2 : Effect of 100ug/ml of Histamine on rabbit ileum

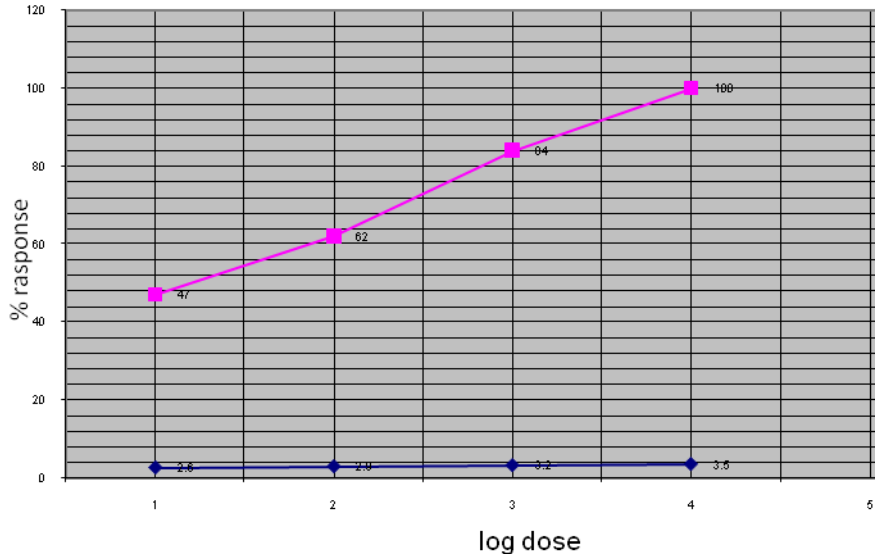

Chart 3: Effect of 5ug/ml atropine on rabbit ileum

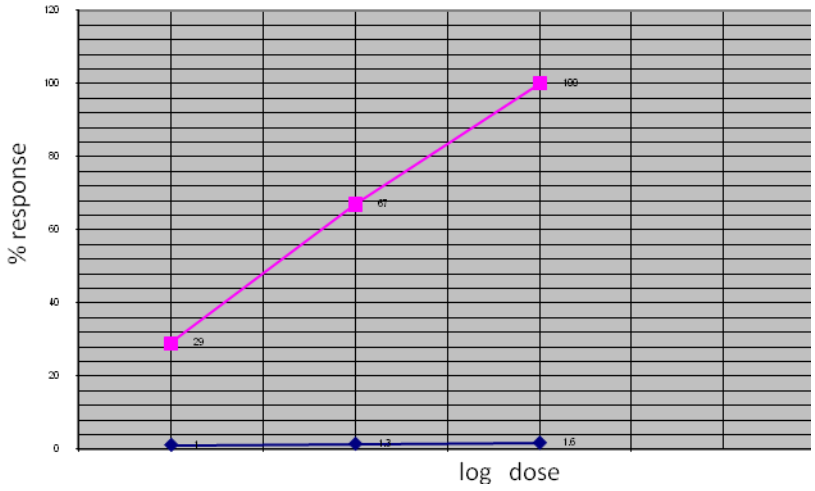

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Chart 4: Effect of 5ug/ml Adrenaline on rabbit ileum

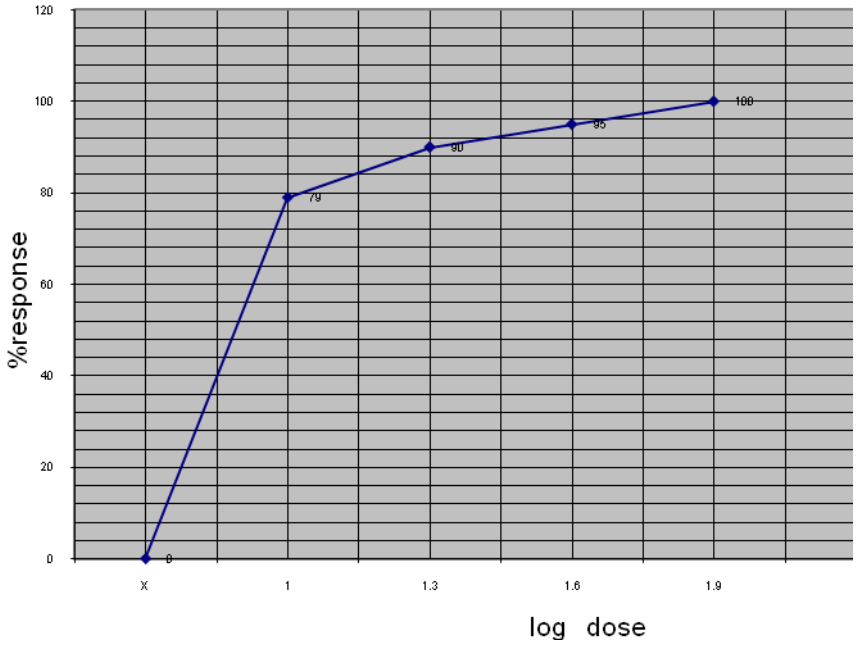

Chart 5 : Effect of 10mg/ml of fraction 'AC' on rabbit ileum

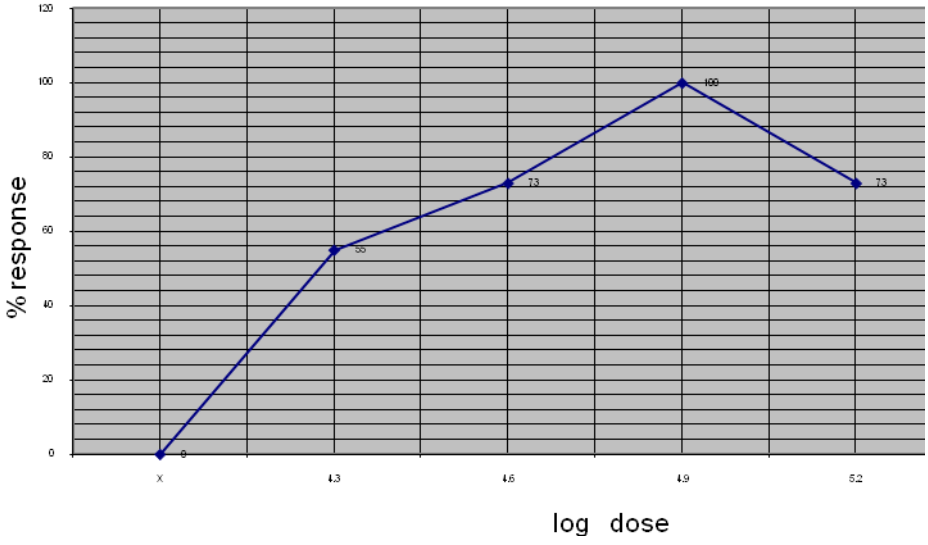

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Chart 6 : Effect of 10mg/ml of fraction 'CC' on rabbit ileum

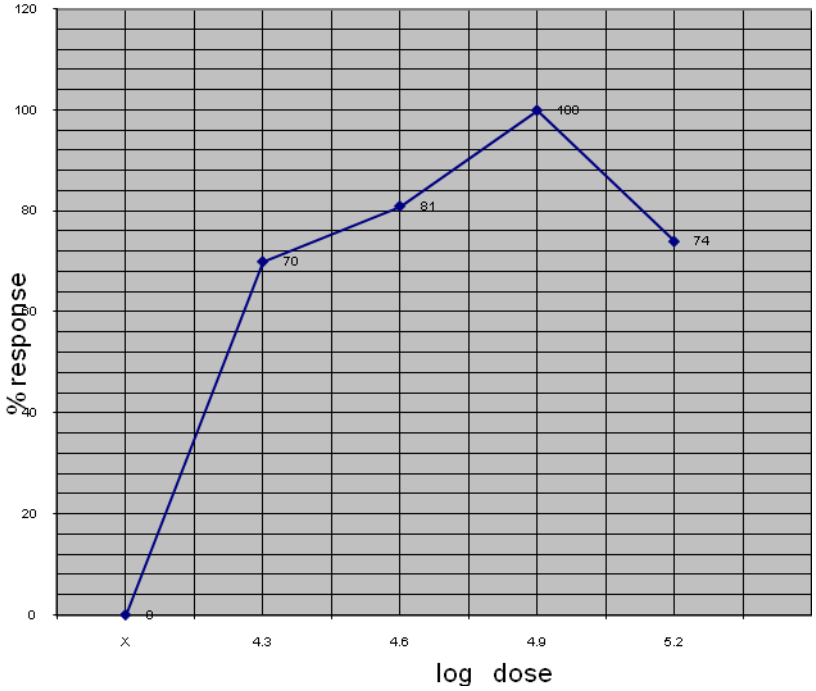

Chart 7: Effect of 10mg/ml of fraction 'EA' on rabbit ileum

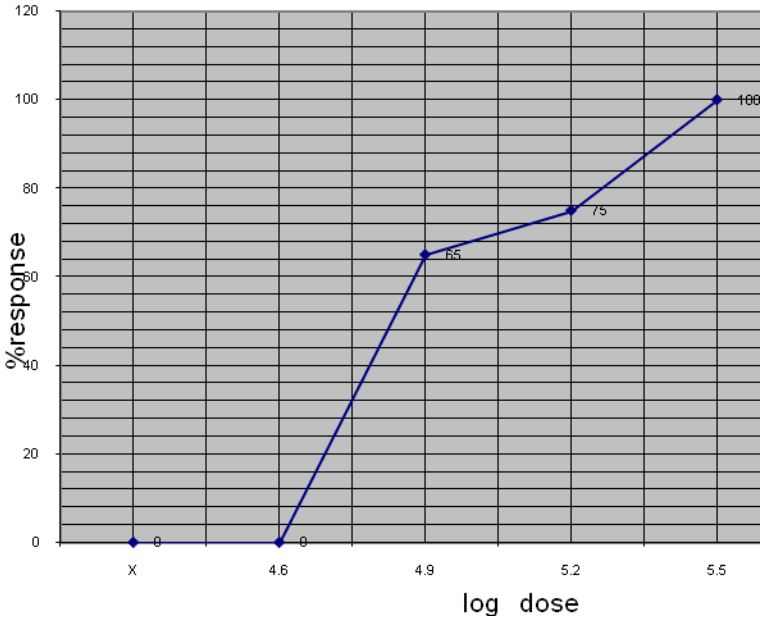

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Chart 8 Effect of varying dose of 5ug/ml Ach against 0.02 ug/ml atropine

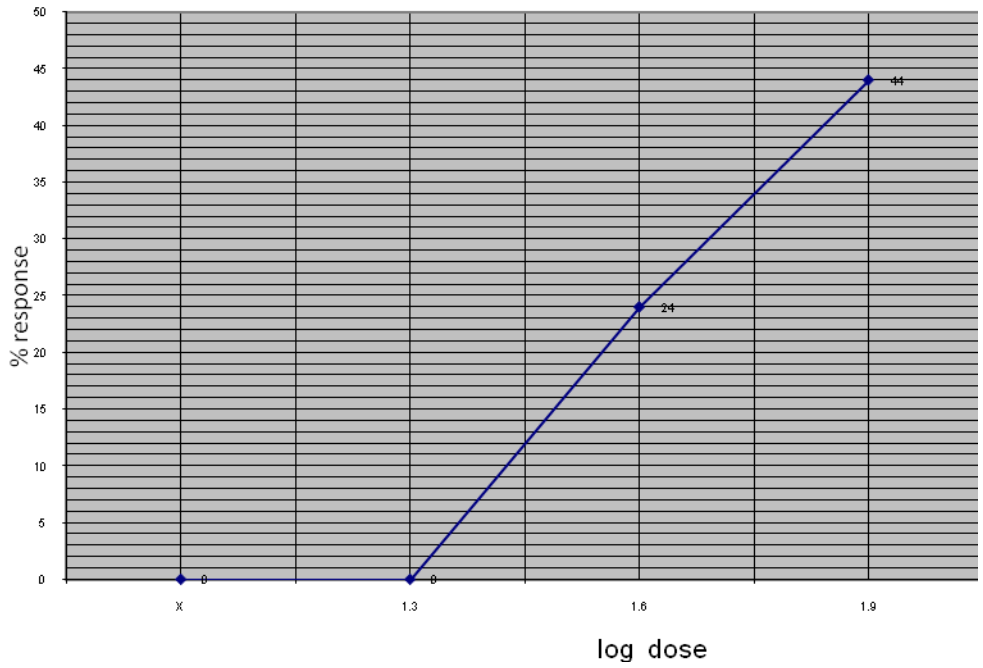

Chart 9 : Effect of varying dose of 10mg/ml of fraction 'CC' against 0.04ug/ml Ach

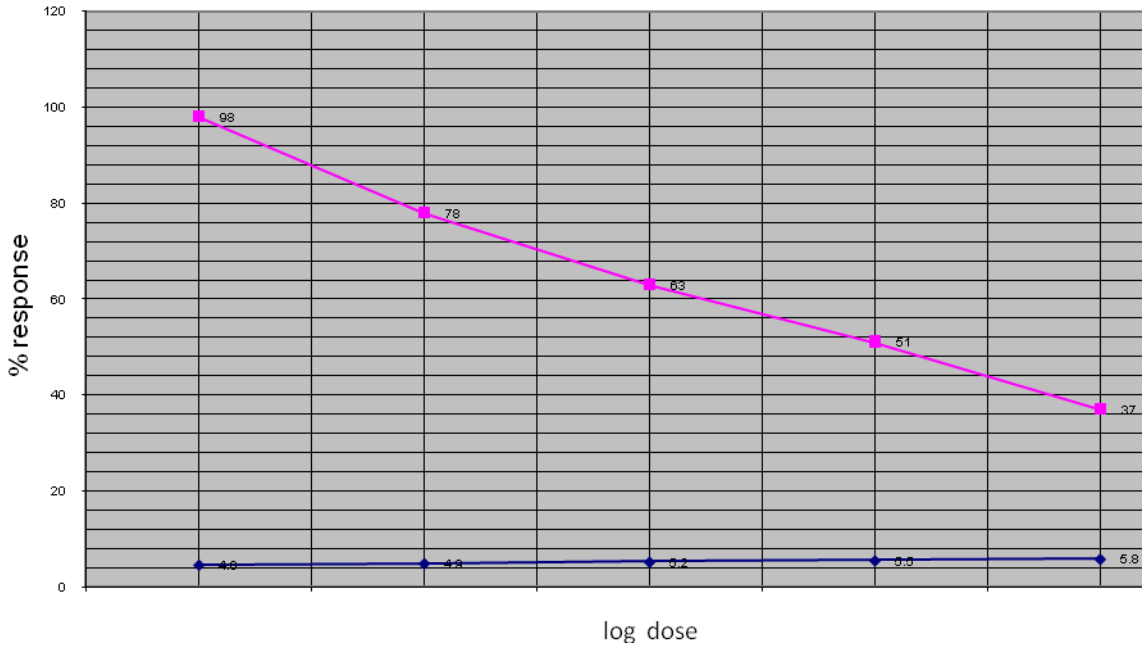

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Chart 10 : Effect of varying dose of 10mg/nl of fraction 'EA' against 0.04ug/ml 0f Ach

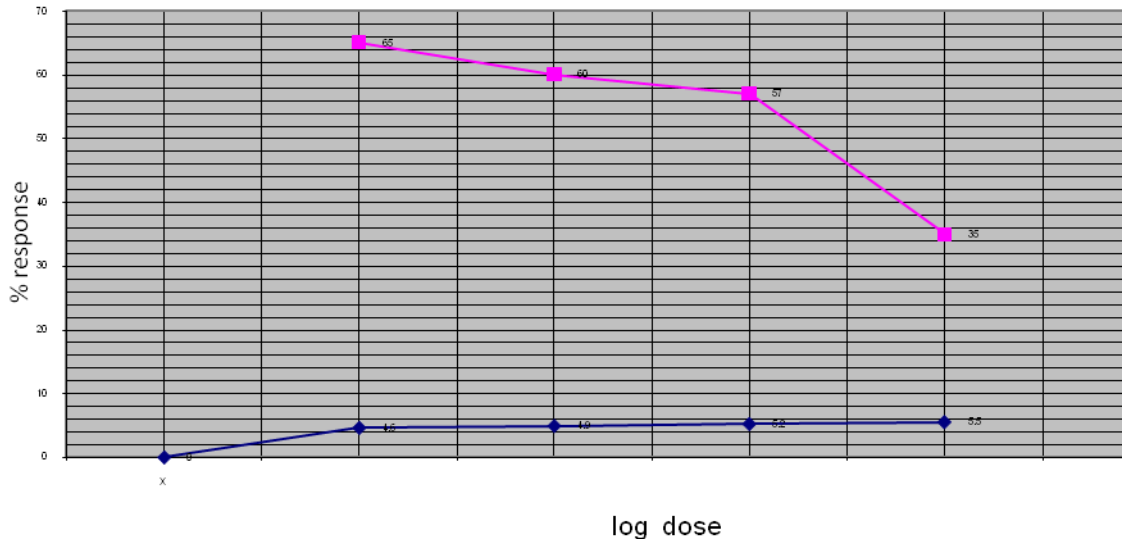

Chart 11 : Effect of varying dose of 10mg/ml of fraction 'CC' against 0.2ug/ml of Histamine

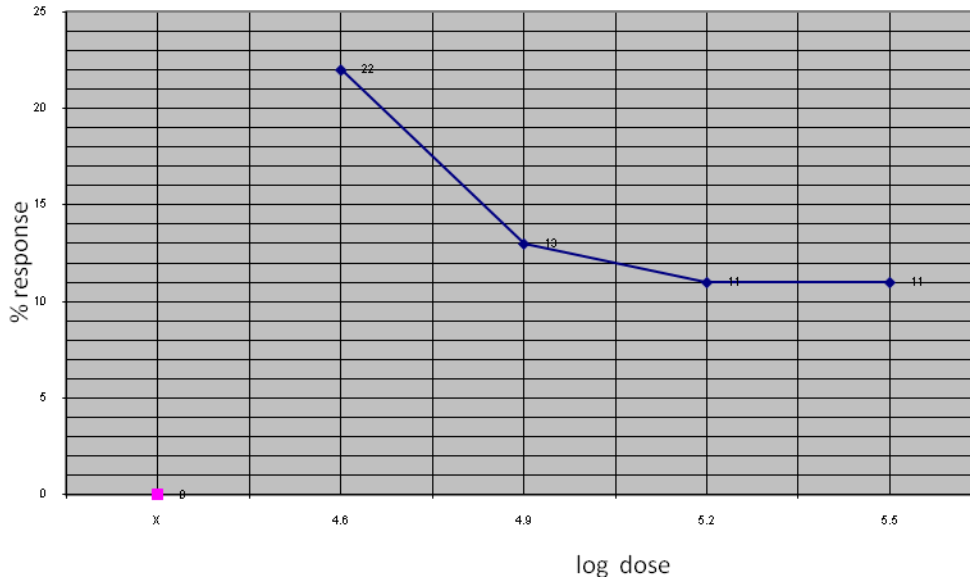

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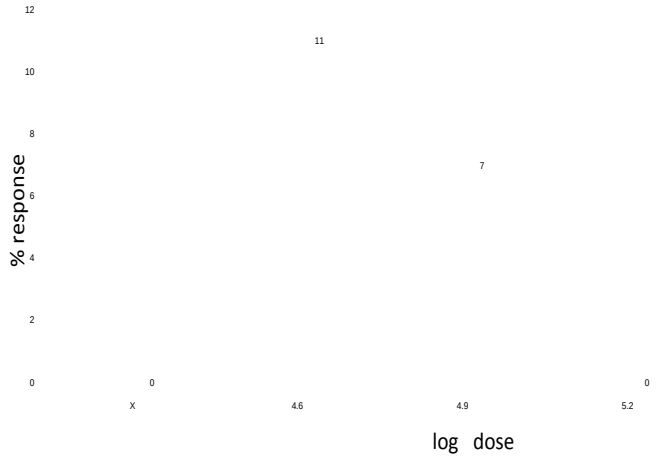
Chart 12 : Effect of varying dose of 10mg/ml of fraction 'EA' against 0.2ug/ml of Histamine



DISCUSSION

Rabbit intestine produce spontaneous pendular contraction, drugs that cause contraction or relaxation effect on the smooth muscle, could be identified easily by their response on the intestinal smooth muscle.

As expected 5μg/ml acetylcholine and 100μg/ml histamine (Table 1/2) produce dose dependent contraction on the isolated rabbit and guinea pig ileum while 5μg/ml Atropine and 5μg/ml Adrenaline (Table 3/4) produces dose dependent relaxation on the isolated rabbit ileum and their responses are used as standard. Fraction 'CC', 'EA' and 'AC' of leave of Cycas circinalis produce a dose dependent relaxation on the rabbit ileum (Table 5/6/7) at concentration of 10mg/ml from 0.1ml to 0.4ml similar to that of Atropine and Adrenaline. The maximal interacting effect of Atropine on isolated tissue was observed with various doses of Ach (Table 8) at a concentration of 5μg/ml of Ach from 0.1ml to 0.4ml. Interactive effect of various dose of 10mg/ml fractions 'CC' and 'EA' from 0.1 to 1.6 against 0.2ml 5μg/ml Ach (Table 9 / 10) to observe inhibition of fractions 'CC' and 'EA' on Ach. Also interactive effect of various dose of 10mg/ml fractions 'CC' and 'EA' from 0.1 to 0.8 against 0.2ml 100μg/ml Histamine (Table 11/12) to observe inhibition of Histamine induce contraction by fractions 'CC' and 'EA'.

Percentage response and log dose response graph of both reference drugs and fractions, on rabbit ileum were plotted (Chart1-7). Log does response graph of the interaction are shown (Chart 8-12). In this the log dose response of Atropine, Adrenaline and the fractions are similar (Chart3-7). However those of the fractions were shifted to the right and parallel to that of Atropine (Chart5, 6,7 and 3) indicating that they have similar mechanisms of action. This also shows that they are less potent than atropine.

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Since atropine and other cholinergic antagonist blocks the colic and spastic action brought about by Ach on intestinal muscle [7]. They are therefore beneficial in colic spasm and increase in sparastalsis. Fractions 'AC', 'CC' and 'EA' of *Cycas circinalis* leaves also antagonize Ach induced contraction on isolated rabbit and guinea pig ileum so they have anticholinergic activities. Since anticholinergy cannot be as purgative hence the pharmacological action shows the leave could then be a synergistic part (synergism).

CONCLUSION

The result in this case doe's not agree with the traditional use of the plant as a purgative. But the leave could be a synergistic part (synergism). It is in this vein suggested that further work should be carried out to establish if the traditional use and its result is psychological or other constituent from the ingredient apart from the leaves are responsible for the observed actions.

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