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HYPOTENSIVE, SPASMOLYTIC AND SPASMOGENIC EFFECT OF CYPERUS ROTUNDUS CRUDE EXTRACT AND ITS FRACTIONS

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ABSTRACT

The main objective of present study is to explore the hypotensive and GIT effect of traditionally important plant *Cyperus rotudus*. Aqueous extract of *C. rotundus* caused a decrease in mean arterial blood pressure in anaesthetized Sprague-Dawley rats in a dose dependant manner. At the dose of 3 mg/kg the MAB was found to reduce by 42.6% from its control. This fall in the MABP was statistically significant ($p<0.0005$). The increased dose of *C. rotundus* (10 and 30 mg/kg) showed a less reduction in the MABP (22.3% and 10.4% respectively). The crude extract and its fractions (ethylacetate, chloroform, *n*-butanol and aqueous) were analyzed for its spasmogenic and spasmolytic activity *in vitro*, on rabbit intestine and the effects were compared with standard drugs acetylcholine, adrenaline and atropine. The crude extract of *C. rotundus* exhibited highly significant (70.16%) relaxing action on smooth muscles in comparison to control and standard drugs. When it was fractionated except ethylacetate fraction which showed spasmolytic action all other fraction showed very strong spasmogenic activity (chloroform 83.87%, *n*-butanol 77.11% and aqueous 96.5%). It was also observed that spasmolytic activity was dominant in crude extract.

Keywords: *C. rotundus*, Hypotensive, Spasmolytic, Spasmogenic

INTRODUCTION

Cyperus rotundus is effective in different ailments and disease [1]. It was evaluated to explore its hidden properties on scientific bases. It is a common weed found in central southern India and other parts of the worlds. Tuber of this plant is useful in fever, diarrhea, vomiting, cholera, dysentery and dyspepsia. It is also used as a galactagouge, skin and hair caring products and relaxant aromatherapy [2]. Beside these effects, different scientist also reported that it is a stimulant, diuretic, anthelmintic, stomachic, astringent, emmenagogue. Essential oil from tuber showed estrogen activity while volatile oil from tuber exhibited antimicrobial effect [2-4].

Hpypertension and Gastrointestinal problems [5,6] can be the risk of severe disorders. Hypertension is one of the leading cause of sudden death. Similarly diahrroea and other GIT complain also requires serious attention. The world wide increasing demand for medicines in these disorders has motivated search for drugs from natural origin with potential beneficial effect. Therefore, besides exploring the new pharmacological activities of *C. rotundus*, one of the aspects of present study is to provide a useful remedy

for the treatment of high blood pressure and GIT problem.

MATERIALS AND METHODS

Cyperus rotundus (tuber ethanol extract) was used in the present study [1]. 10g-15g of crude extract was further fractionated to ethylacetate, chloroform, *n*-butanol and aqueous fractions [7-8].

Animals: Sprague-Dawley rats of either sex (200-250g) and rabbits (1-1.5 kg) were used for effect on blood pressure and smooth muscle activity respectively. They were kept at the animal house of Dr. HMI Institute of Pharmacology and Herbal Sciences, Hamdard University, in ambient environment .They were given standard food and water ad-libitum. The ethical permission was taken from Dr. HMI institute before these experiments.

Measurement of Mean Arterial Blood Pressure (MABP): Mode of administration was intravenous. Three doses (3mg/kg, 10mg/kg and 30mg/kg) of *C. rotundus* were prepared by decoction in aqueous base for experimental use. Acetylcholine (I0-4M) was used is a positive control. Pentothal sodium Abbott laboratories, Karachi, Pakistan, used as anesthetic. All these drugs were dissolved in distilled water. Saline (0.9% NaCI), Heparin (Leo Pharmaceutical Denmark) was used to prevent clotting [9].

Experimental Procedure: Normotensive Sprague-Dawley rats of either sex were anesthesized wtth pentothal sodium (50mg/kg IP) Spontaneous respiration of anesthesised animal was maintained by inserting tracheal cannula after tracheotomy. The left juglar vein was cannulated with polyethylene tube for intravenous administration of drugs and flushing of 0.9% NaCl. A polyethylene cannula filled with heparin was placed into right carotid artery for the purpose of arterial blood pressure measurements. The other end of this cannula was connected to Research grade blood pressure transducer (Harvard, 60-3003). This transducer was then connected with four channel Harvard universal oscillograph (curvilinear, 50-9307) for recordings. The temperatur of the animal was maintaines at 37°C by use of overhead lamp, animals were always left to equilibrate for a period of 20-25 minutes before recording [9].

Measurements: Mean arterial blood pressure was calculated as diastolic blood pressure. Changes in blood pressure were expressed as the percent of control values, obtained immediately before the administration of test substance [9].

Smooth muscle activity: Rabbits were sacrificed (blow on the back neck) and the abdomen was made open immediately. The intestine was then cut from animal and placed in a beaker containing Tyrode's solution [10-12]. The temperature of Organ bath was maintained to 37oC and Tyrode solution was perfused with a mixture of 95% oxygen and 5% carbondioxide. The spontaneous movements of intestine were recorded on oscillograph. To determine the effects of crude extract and its fractions were dissolved in 1 ml of distilled water [10]. The changes in rhythmic pattern of rabbit's intestine were recorded at different concentrations of crude extract and fractions and compared with control and standard drugs (Acetylcholine, Atropine and Adrenaline) [13].

Statistical analysis: The data was analyzed by using standard statistical tools i.e. mean, standard error and percent control. The comparison of the average value of various parameters obtained from control and *C. rotundus* treatment was done by using student's t test. The significance was considered at p level of 0.05 and 0.0005

RESULTS

Effect of the crude extract of Cyperus rotundus on the MABP in rats: Aqueous extract of *C. rotundus* caused a decrease in mean arterial blood pressure in anesthesized Sprague-Dawley rats in a dose dependent manner (Figure 46-47). At the dose of 3 mg/kg the mean arterial blood pressure was found to reduce by 42.6% from its control. This fall in the mean arterial blood pressure was statistically significant ($p<0.0005$). An increase in the dose of *C. rotundus* i.e. 10mg/kg and 30mg/kg showed a less reduction in the mean arterial blood pressure 22.3% and 10.4% respectively. The reductions in MABP was also statistically significant (Table1).

Effect of the crude extract and fractions of C. rotundus on the isolated rabbit intestine: This activity was carried out on isolated intestine of rabbit and spontaneous movements of intestine were recorded on Oscillograph using isotonic transducer. The crude extract of *C. rotundus* and its fractions (ethylacetate, chloroform, *n*-butanol and aqueous) were analyzed at different concentrations (Table 2-3 and figure 2-3).

Maximum spasmolytic response observed at the concentration/dose of 25mg/ml (70.24%) of crude extract of *C. rotundus*. Ethylacetate fraction of *C. rotundus* showed decrease in smooth muscle activity (53.30%). Where as chloroform (83.87%), *n*-butanol (77.11%) and aqueous (96.55%) fractions exhibited

spasmogenic response. The fractions of *C. rotundus* were also analyzed with standard drugs atropine, acetylcholine and adrenaline (Graph1-4).

DISCUSSION

Hypertension is a serious risk factor for cerebro-vascular disease and heart diseases. It is commonly accepted that antihypertensive therapy improves the quality of life of the patients. Although there are several antihypertensive drugs clinically available, but due to the different origins and pathologies of hypertension, it is difficult to control all types of hypertension through the use of only one drug, and each antihypertensive agents possess their side effects [14].

Essential hypertension continues to be a contributing factor to the morbidity and mortality associated with cardiovatcular diseases. Despite a large number of drugs available for the treatment of hypertension, heart diseases and peripheral and cerebral vascular complications continue to occur [15]. Various cardiotoxic sequelae have been attributed to exaggerated sympathetic activity, including chronic heart failure, atherosclerosis, angina, and cardiac arrhythmias [15].

It is generally accepted that arterial blood pressure depends on cardiac output and vascular resistance. The physiological processes related to vascular function differ greatly between resistance arteries and conduit arteries. Large arteries such as the aorta are considered as passive conduits with the principal action of conducting and distributing cardiac output to various tissues. Resistance arteries but not conduit arteries are important for the regulation of both regional blood flow and vascular resistance [16]. The aim of the present work was to study the effects of *C. rotundus* on blood pressure and the mechanism on the hypotensive effects of this drug. For that purpose, we have investigated the effects of *C. rotundus* on blood pressure parameters and heart rate in the anesthesised and normotensive rats. The blood parameter has not been used before for evaluation and determination of the effects of extract of *C. rotundus* in Sprague-Dawley rats.The worldwide increasing demand for medicines from natural sources[17] has motivated search for drugs with potential hypotensive activity as well.

Aqueous extract of *C. rotundus* caused a decrease in mean arterial blood pressure in anaesthetized Sprague- Dawley rats in a dose dependent manner. At the dose of 3mg/kg the mean arterial blood pressure was found to reduce by 42.6% from its control. This fall in the mean arterial blood pressure was statistically significant ($p<0.0005$). An increased in the dose of *C. rotundus* i.e. l0mg/kg and 30mg/kg showed a less reduction in the mean arterial blood pressure 22.3% and 10.4%, respectively, the reductions in MABP were also statistically significant (Table 1 figure 1a,b,c).

Physiologically, reduction in blood pressure has been reported to be associated with either decrease in peripheral resistance directly or maintained through central nervous system[18]. On the basis of above presented results it is difficult to pin point exact site for hypotensive effect of *C. rotundus*. However, it is hypothesized that this drug might be acting peripherally[19] by relaxing (smooch muscles) arteriolar wall, thus, reducing the peripheral resistance [18]. However, its action through CNS cannot be ruled out which may be investigated by involving receptor identification and probable effects of *C. rotundus* on medullary cardiac centers [20].

The maximum reduction in the mean arterial blood pressure observed at 3mg/kg dose indicate that this dose can be regarded as optimal dosage for hypotensive aciivity in rats. According to Guyton (1996) changes in systolic and diastolic blood pressure are not usually of same magnitude in different physiological conditions as well as under the influence of various drugs [21].

Spasmogenic and spasmolytic acitivity is a reliable test for the detection of anitdiarrhoeal activities of a drug. This drug is also used in the treatment of diarrhea and dysentery [2]. Woo and Lee (1976) reported that methanol extract of rhizome, at a concentration of 1mg/ml was found inactive on rat ileum [22]. The crude extract of *C. rotundus* exhibited highly significant (70.16%) relaxing effect on smooth muscle of rabbit intestine. But when it was fractionated with ethylacetate, chloroform, *n*-butanol and aqueous fractions, except ethylacetate fraction (spasmolytic) all other fractions showed strong spasmogenic response. It was also observed that spasmolytic activity was dominant in crude extract except 1mg/ml concentration which showed initial contraction (5.85%). The fractions of *C. rotundus* were also treated with standard drugs atropine, acetylcholine and adrenaline (at $1x10^{-2}$ M, $1x10^{-4}$ M concentrations) to find out the possible mechanism of action. Pretreated tissue with adrenaline ($1x10^{-4}$ M) did not alter the spasmolytic response of *n*-butanol fraction however; at high conc. this response was inhibited. Similarly pretreated tissue with acetylcholine $1x10^{-2}$ M in *n*-butanol (Graph 2) and chloroform (Graph 3) fractions exhibited synergistic effect. In case of aqueous fraction (Graph 4)

pretreated tissue with atropine $1x10^{-2}$M conc. did not produces spasmogenic response hence showed the possible involvemt of muscarinic receptors. Post and pretreated tissue with acetylcholine $1x10^{-2M}$ and $1x10^{-4}$M conc. not produces significant classical contraction with ethylacetate fraction (Graph 1). These results suggested that the drug *C. rotundus* has antidiarrhoeal effect probably through muscarinic receptors [23].

CONCLUSION

On the basis of this study it is suggested that *C. rotundus* is probably acting both centrally and peripherally to produce changes in blood pressure by altering the peripheral resistance and cardiac mechanics. It also produces antidiarrhoeal effect by possible involvement of muscarinic receptors. It is therefore concluded that *C. rotundus* is an active drug both physiologically and pharmacologically.

Table 1: Effect of *C. rotundus* on different mean arterial blood pressure parameters

Dose	MABP	
	Control	Treated
3mg/kg	128.91±5.39 (8)	74.07±6.25* (8)
10mg/kg	133.41±2.72 (8)	103.49±6.67 (8)
30mg/kg	138.82±5.25 (6)	124.99±11.7 (8)

All values are presented as mean ±SEM; (n)

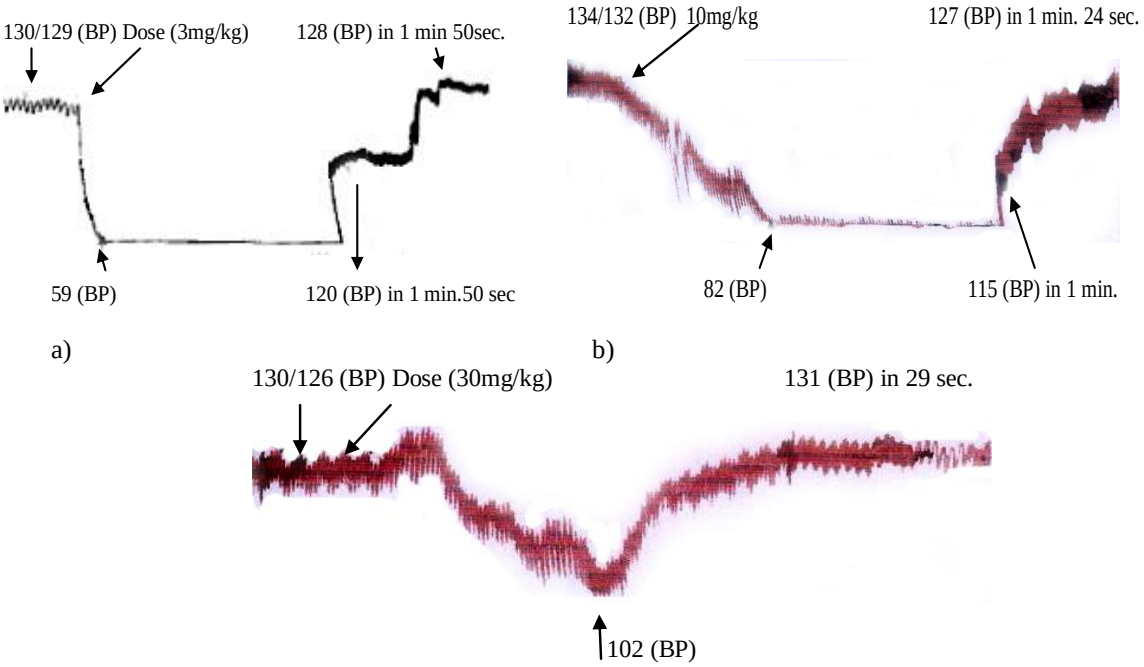


Figure 1(a,b,c): Tracing of crude extract of *C. rotundus* on blood pressure at different doses

Table 2: Dose related response of Crude extract of *C. rotundus* on rabbit's intestine

Dose mg/ml	Control (cm)	Response (cm)	Response in %	t-value
1	0.63±0.09	0.67±0.03	5.85	0.46
5	1.03±0.03	0.63±0.03	38.71	14.14
10	1.13±0.03	0.83±0.03	26.50	12.5
15	0.93±0.07	0.50±0.06	46.41	7.04
20	1.07±0.03	0.47±0.07	56.22	12.3
25	1.23±0.03	0.37±0.07	70.24	29.4

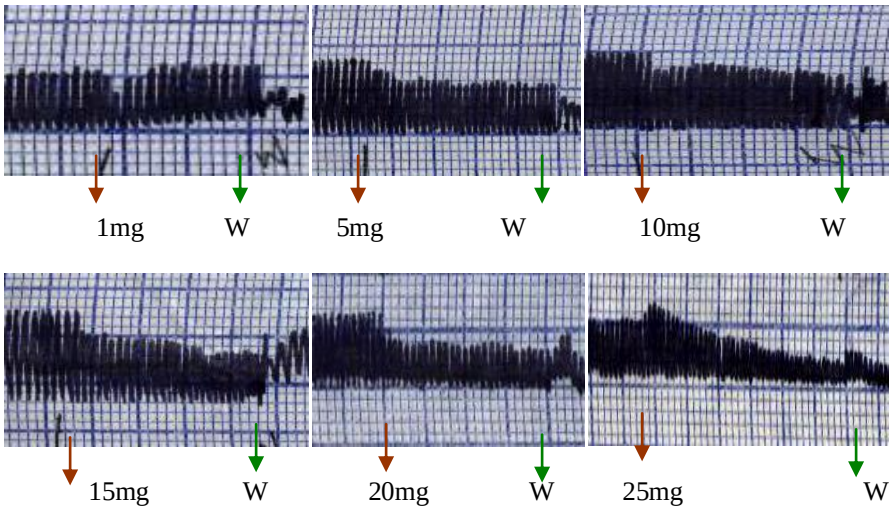


Figure 2: Tracing showed the effect of crude extract of *C. rotundus* at different doses on isolated rabbit intestine

Table 3: Effect of different fractions (at 10 mg/ml) of *C. rotundus* on isolated rabbit's intestine

Fractions	Control (cm)	Response (cm)	Response in %	t-value
Ethylacetate (Spasmolytic)	1.0±0.12	0.46±0.09	53.30	5.38
Chloroform (Spasmogenic)	1.03±0.03	1.90±0.06	83.87	-38.13
n-butanol (Spasmogenic)	0.83±0.12	1.47±0.03	77.11	-5.08
Aqueous (Spasmogenic)	0.97±0.03	1.90±0.03	96.5	-40.13

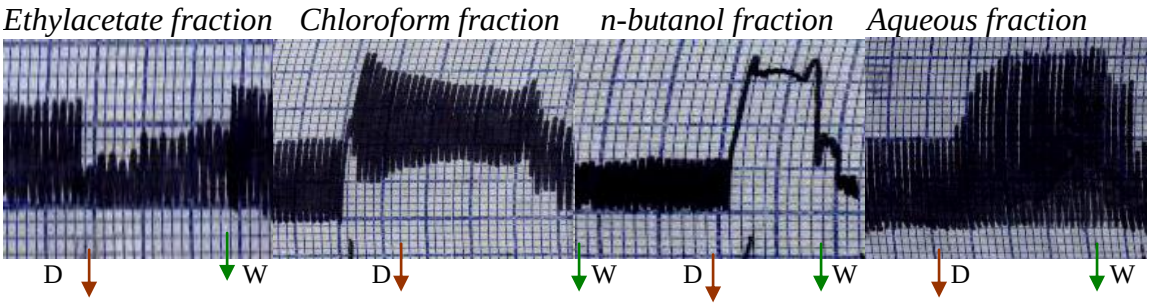
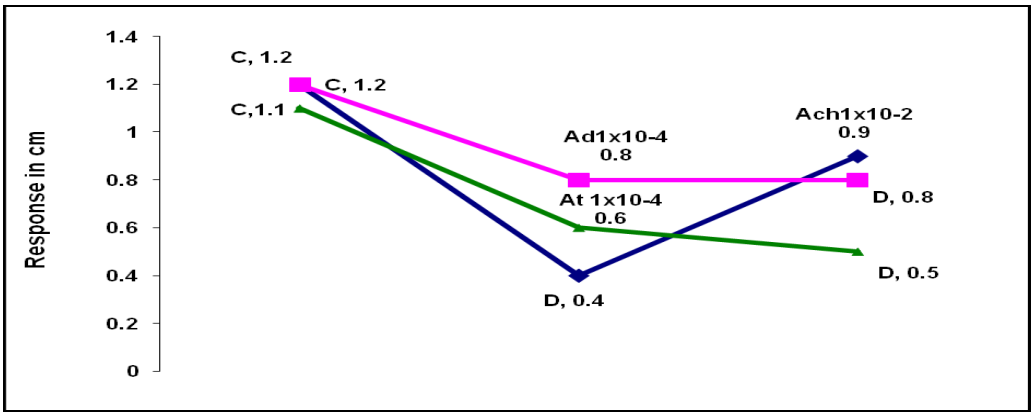
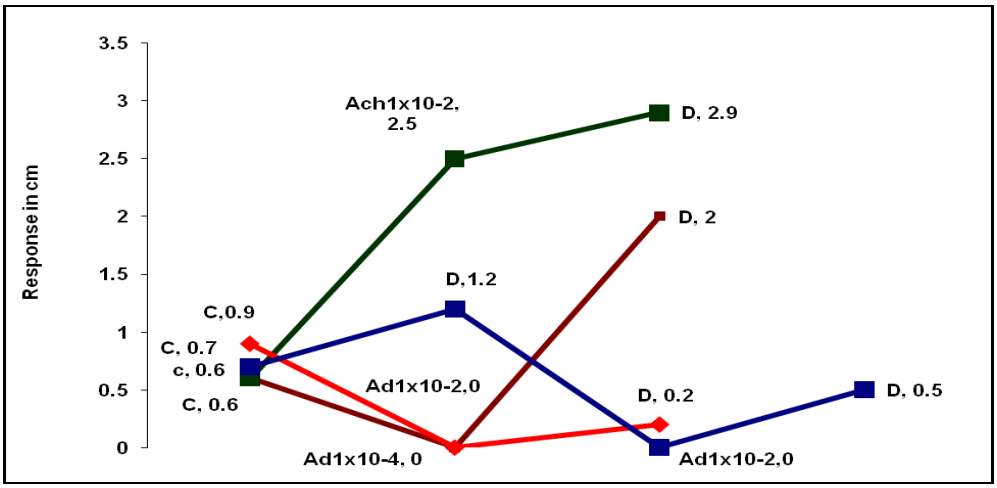


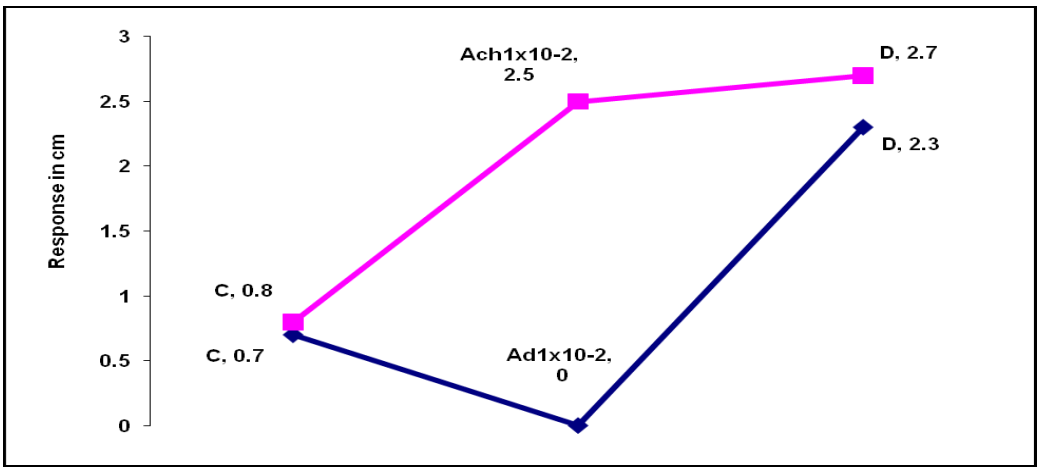
Figure 3: Tracing showed the response of fractions of *C. rotundus* fractions on isolated rabbits's intestine



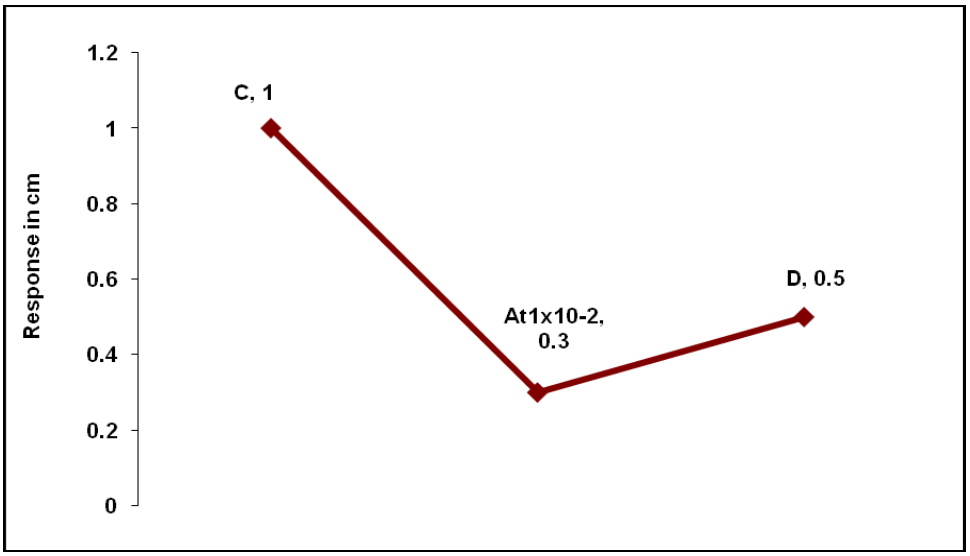
Graph 1: Effect of ethylacetate fraction of *C. rotundus* post and pre treated with atropine, adrenaline and acetylcholine at different conc. (C=control, D= dose 10mg/ml, Ad =adrenaline, At =atropine, Ach = acetylcholine)



Graph 2: Effect of *n*-butanol fraction of *C. rotundus* post and pre treated with atropine, adrenaline and acetylcholine at different conc. (C=control, D= dose 10mg/ml, Ad =adrenaline, At =atropine, Ach = acetylcholine)



Graph 3: Effect of *chloroform* fraction of *C. rotundus* post and pre treated with atropine, adrenaline and acetylcholine at different conc. (C=control, D= dose 10mg/ml, Ad =adrenaline, At =atropine, Ach = acetylcholine)



Graph 4 : Effect of Aqueous fraction of *C. rotundus* post and pre treated with atropine, adrenaline and acetylcholine at different conc. (C=control, D= dose 10mg/ml, Ad =adrenaline, At =atropine, Ach = acetylcholine)

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