

## PHARMACOLOGICAL ACTIONS OF CARDIPRO ON CARDIOVASCULAR SYSTEM

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**ABSTRACT:** CARDIPRO is a polyherbal anti-oxidant and cardiotoxic formulation recommended to be used in various cardiovascular disturbances, effect of CARDIPRO on different *in vitro* and *in vivo* cardiovascular responses were studied. CARDIPRO produced hypotensive effect in dogs and rabbits. The effect was blocked by prior atropinization, CARDIPRO also potentiated and prolonged the beta receptor mediated hypotensive action of adrenaline in dogs. CARDIPRO was found to produce peripheral vasodilation in perfused frog blood vessels, CARDIPRO exerted positive inotropic and negative chronotropic effects on perfused amphibian and mammalian heart (isolated and *in situ*) it also produced transient positive inotropic effect on hypodynamic frog heart. CARDIPRO enhanced the amplitude of contractive response of isolated rat auricle. CARDIPRO did not produce an *per se* effect on electrocardiogram of rabbits, CARDIPRO enhanced both basal and maximum coronary perfusion flow in isolated perfused rabbit heart. The cardiotoxic property of CARDIPRO along with its peripheral and coronary vasodilating effects may greatly help in management of cardiovascular diseases.

### INTRODUCTION

CARDIPRO is a polyherbal antioxidant formulation recommended to be used in various cardiovascular disturbances, hyperlipidaemia etc, the product was proved non-toxic following thirty days administration in rats (1). The constituent plant materials of CARDIPRO (*viz Emblica officinalis, Boerhaavia diffusa, Withania somnifera, Ocimum sanctum* and *Terminalia ajuna*) were reported to possess cardiotoxic, hypotensive, antianginal and hypolipidaemic actions (2-7) the present study was conducted to assess various cardiovascular effects of CARDIPRO *in vitro* and *in vivo*.

### MATERIALS AND METHODS

#### Effect on Blood pressure

Mean arterial blood pressure of anaesthetised dogs and rabbits was recorded via arterial cannula from common carotid artery through a mercury manometer on a

rotating smoked drum connected to a kymograph. The test and reference substances were infused into the femoral vein of dogs and marginal ear vein of rabbits through a polyethylene cannula placed *in situ*. Blood clotting was prevented by injecting heparin (500 U/Kg, *i.v*) at the beginning of the experiment.

#### Effect on perfused blood vessels of frog

The frogs (*Rana tigrina*) were pithed and heart was exposed. One of the aorta was tied and the other cannulated and connected to a reservoir containing frog Ringer solution. A venous cannula was inserted in inferior vena cava to record the number of drops of perfusate coming out the perfusion flow from the reservoir was adjusted in such a manner that the number of drops coming out remained constant at 30 drops / 30 sec. (8). Test substance was administered through aorta and the number of drops coming out from vena cava were counted for 30 sec each

for 6 times. Successive administrations of CARDIPRO were made only after the number of drops returned to control value.

### **Effect on frog's heart (in situ)**

Frogs were pithed and pinned on a frog board. A midline incision was made on the abdomen. The pectoral girdle was removed to expose the heart. Pericardium was removed carefully. A venous cannula was inserted into the inferior venacava which in turn was connected to a perfusion bottle containing frog ringer. A small cut was made in one of the aorta for the perfusate to come out. Constant pressure was maintained by adjusting the height of perfusion bottle. A pin-hook was then passed through the tip of the ventricle and with the help of a fine silk thread, it was attached with a universal lever connected to a smoked drum of kymograph. 1 gm tension was applied to the heart. Amplitude and rate of cardiac contraction were recorded after injecting the product into the perfusion tube close to the venous cannula taking every caution to avoid any leakage of the test substance from the tube.

### **Effect on isolated perfused frog's heart**

Frogs of 100 gm weight were used for this experiment. These were pithed and subjected to thoracotomy. The heart was removed and attached to Langendorff's apparatus in a retrograde manner. Frog's Ringer solution was used at room temperature without oxygenation as perfusion fluid. Cardiac contraction was recorded by an isotonic transducer attached to the apex of the isolated heart through a silk thread. The heart was loaded to a resting tension of 1 gm. The transducer was connected to a physiograph where contraction of isolated heart was recorded.

### **Effect on isolated perfused rat's heart**

Contractile response of isolated perfused rat's heart was recorded as stated above in frog's heart. In this experiment, the heart was suspended in a temperature controlled chamber and perfused with warm (37°C) Ringer's solution continuously bubbled with 95% oxygen / 5% carbon-dioxide. (9)

### **Effect on isolated perfused Hypodynamic rat/frog heart**

Contractile response of isolated hypodynamic rat and frog heart was recorded similarly as above using one-fourth calcium in the perfusion fluid.

### **Effect on isolated rat auricle**

Isolated rat auricle was prepared as described elsewhere (10). Adult wistar rats were subjected to thoracotomy under urethane anaesthesia. The intact heart was removed and put into Feigán's solution at room temperature. The pericardium was removed.

The ventricular tissues were dissected away taking care not to damage the S-A node. A thread was tied round the tip of each auricle. It was mounted in a 4 ml organ bath containing Feigán's solution through which oxygen was bubbled at room temperature. Auricular contraction was recorded by a physiograph connected through an isotonic transducer.

### **Effect on Electrocardiogram (ECG) of rabbits**

Adult albino rabbits (1.5 kg) tranquilized with trifluoromazine (1mg/kg; i.v.) were used for this experiment. The electrodes constructed from 26 gauge hypodermic needles were placed subcutaneously 1 cm

deep into the four limbs. To avoid electrical interference during recording, the rabbits were placed on wooden plank and were earthed. Standard limb lead II was used to record ECG by a physiograph. Sensitivity was adjusted to provide a deflection of 30mm for 1 mv standard square wave. Different doses of CARDIPRO were administered through marginal ear vein.

### **Effect on coronary perfusion flow**

Isolated rabbit heart was mounted on Langendorff apparatus through the aorta and perfused in a retrograde manner with a perfusion pressure of 50mm Hg. The perfusion fluid (Ringer's solution) was oxygenated and maintained at 37°C. Immediately after mounting, the heart was perfused and allowed to equilibrate for 30 min. The pulmonary arterial effluent was time collected for determination of coronary flow (11). The baseline coronary flow (ml/min/g wet heart weight) was measured after equilibration period.

The maximum coronary flow (ml/min/g) which represented the maximum vasodilation response to reactive hyperaemia was measured following a 45sec occlusion of the inlet flow. The maximum total coronary flow (ml/min) which was not corrected for cardiac mass was also calculated to serve as an index of total vascular conductance.

## **RESULTS AND DISCUSSION**

CARDIPRO produced significant cardiovascular effect in different in vivo and in vitro experiments.

### **Blood pressure response**

CARDIPRO (1 mg/kg i.v.) produced a fall in mean arterial blood pressure of dogs and

rabbits (Fig.1) It has been reported that some of the constituents of *B. diffusa*, *W. somnifera*, *O.sanctum* and *T. arjuna* produced hypotensive effect in experimental animals (4,6,12-17). It was also observed that the hypotensive response was absent in atropinized (1mg/kg) animals (Fig 2) although the precise mechanism of this type of response is not clear, some cholinergic reaction might be occurring following CARDIPRO administration in experimental animals, presence of cholinergic (muscarinic) active principles in *B. diffusa* and *T.arjuna* was postulated earlier (4,13).

In our present study it was also noted that CARDIPRO (0.5mg/kg) did not block the pressor response of adrenaline (2mcg/kg) rather it potentiated and prolonged the beta-receptor mediated hypotensive action of adrenaline in dogs (Fig 3).

### **Perfused blood vessels**

CARDIPRO produced peripheral vasodilation in perfused frog's blood vessels (Fig-4). CARDIPRO in two different concentrations (100 and 250 mcg) increased the number of drops of outcoming perfusate from venecava through systemic circulation. So, it can be inferred that the hypotensive effect of CARDIPRO, as seen in the present study, is mediated through peripheral vasodilation. Flavonoid compounds have been reported to possess remarkable vasodilator properties (18). Flavobols inhibit the activity of adenosine (IC<sub>50</sub> = 26-32 μM), the enzyme responsible for metabolic degradation of endogenous adenosine. Hypotensive action of flavonoids are mediated by amplification of the effect of endogenous adenosine via adenosine receptors (19). The role of adenosine in regulation of blood pressure is well established (20). The presence of flavonoid compounds as detected by HPTLC analysis

of the aqueous methanolic (1:1) fraction of this product (data not shown) may explain its vasodilating effect.

### **Myocardial contraction**

CARDIPRO (10 and 100 mcg) produced positive inotropic effect on rat heart (isolated) and frog heart (isolated & *in situ*) (Fig 5-7).

Cardiotonic effect of *B. diffusa*, *E. officinalis* and *T. arjuna* was reported previously (3, 7, 12). It was suggested that free amino acid constituents of *B. diffusa* root (leucine, methionine, serine, glutamic acid etc.) produced cardiotonic effect in isolated frog heart (21).

In our present study negative chronotropic effect of CARDIPRO was evident in isolated frog's heart. *B. diffusa* and *T. arjuna* produced similar effect on amphibian and canine heart, respectively (12, 15). The positive inotropic and negative chronotropic action of any product is most desirable to be a therapeutically active and safe cardiotonic agent. CARDIPRO (upto 100 mcg) produced variable effects on hypodynamic frog and rat heart. It did not show any effect on hypodynamic rat heart but produced transient increase in contractile amplitude of hypodynamic frog heart (Fig – 8)

### **Effect on isolated auricle**

CARDIPRO (100 mcg) produced slight increase (20% approx) in amplitude of contraction of isolated rat auricle preparation. Lower dose of CARDIPRO (10 mcg) failed to produce an effect on auricular contraction (Fig 9). It was reported that *T. arjuna* produced positive inotropic effect on auricular contraction (7,22) which was mediated via beta-adrenoceptors. Similar effect was reflected in CARDIPRO.

### **Effect on ECG**

CARDIPRO (upto 1mg/kg) did not produce any *per se* effect on electrocardiogram of rabbits.

### **Coronary perfusion flow**

*In vitro* perfusion of the heart is a suitable approach to explore the efficiency of coronary flow in animals. CARDIPRO at different concentrations (10,50 and 100 mcg) increased the baseline coronary flow and maximum coronary flow (which represents maximum vasodilation response to reactive hyperaemia) in perfused rabbit heart (Table -1).

The maximum total coronary flow which serves as an index for total vascular conductance was also increased in perfused rabbit heart treated with CARDIPRO. The probable mode of action of CARDIPRO on coronary perfusion flow appears to be due to

1. Increased mechanical compression of coronary vessels owing to forceful contraction of the surrounding myocardium
2. Increased myocardial contraction leads to increased myocardial oxygen consumption. Relative myocardial hypoxia causes reflex release of adenosine from cardiac myocytes. Adenosine produces relaxation of coronary arteries and thereby increased coronary perfusion flow (20).
3. Root and *B. diffusa* contains hypoxanthine – 9-L-arabinofurano side which is structurally similar to common nucleosides like adenosine (23). This may also influence the coronary

perfusion flow in CARDIPRO treated perfused rabbit heart.

- Nitrates present in the root of *B. diffusa* may be causing increased coronary perfusion flow.

Our present study confirms that CARDIPRO produces cardiotoxic and hypotensive action. It produced positive inotropic and negative chronotropic effects. Cardiotoxic effect was also confirmed in hypodynamic amphibian heart. To understand the possible mechanism of action of CARDIPRO concerning the effects on blood pressure, perfused blood vessels and myocardial contractility of experimental animals and as

to how it increases the coronary perfusion flow it is necessary to consider the fact that the product contains several pharmacologically active substances. Therefore, a variety of central and peripheral receptors may be involved. The results of the present study indicate that CARDIPRO can be used to treat various cardiovascular disturbances including angina pectoris, after required clinical evaluation.

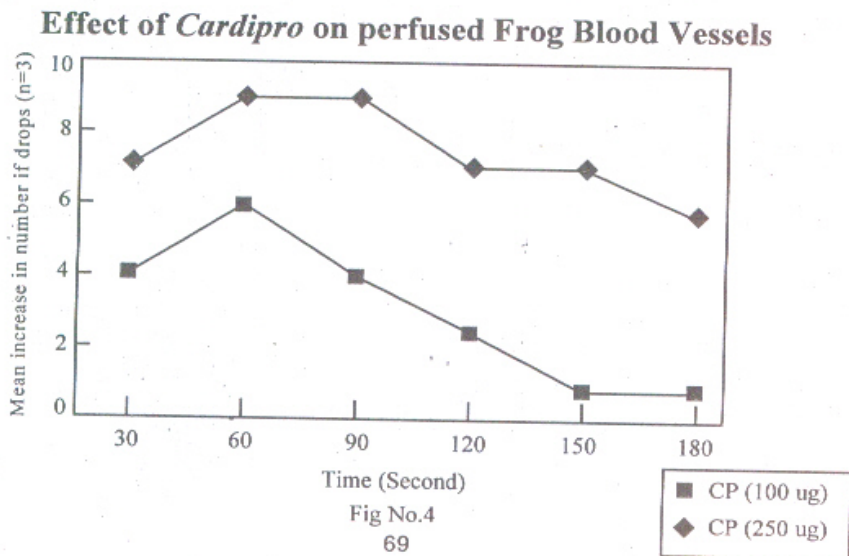
### Acknowledgement

The authors are thankful to Mr. Brijpal (Laboratory chemist) for providing support in HPTLC analysis of the product.

**Table 1**  
**Effect of cardipro on coronary perfusion flow in isolated rabbit heart preparation**

Parameters	Control	Cardipro		
		10µg	50µg	100µg
Coronary flow (ml/min/gm)	4.9±0.3	6.1 ± 0.4	6.3 ± 0.3*	6.7 ± 0.6*
Max. coronary flow (ml/min/gm)	7.2 ±0.5	8.5 ± 0.5	8.9 ± 0.4*	9.2 ± 0.5*
Total max. coronary Flow (ml/min)	36.0 ±3.1	45.5 ± 3.7	47.6 ± 4.7	49.2 ± 4.2

\*P<0.05 as compared to control (n=6)





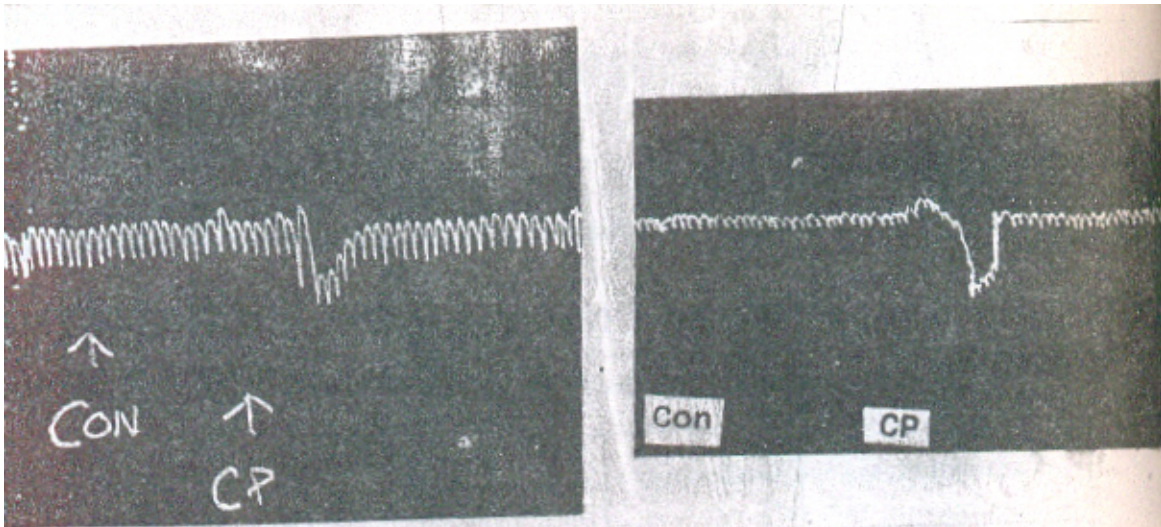


Fig.1. Effect of Cardipro on blood pressure of dog (left panel) and rabbit (right panel).

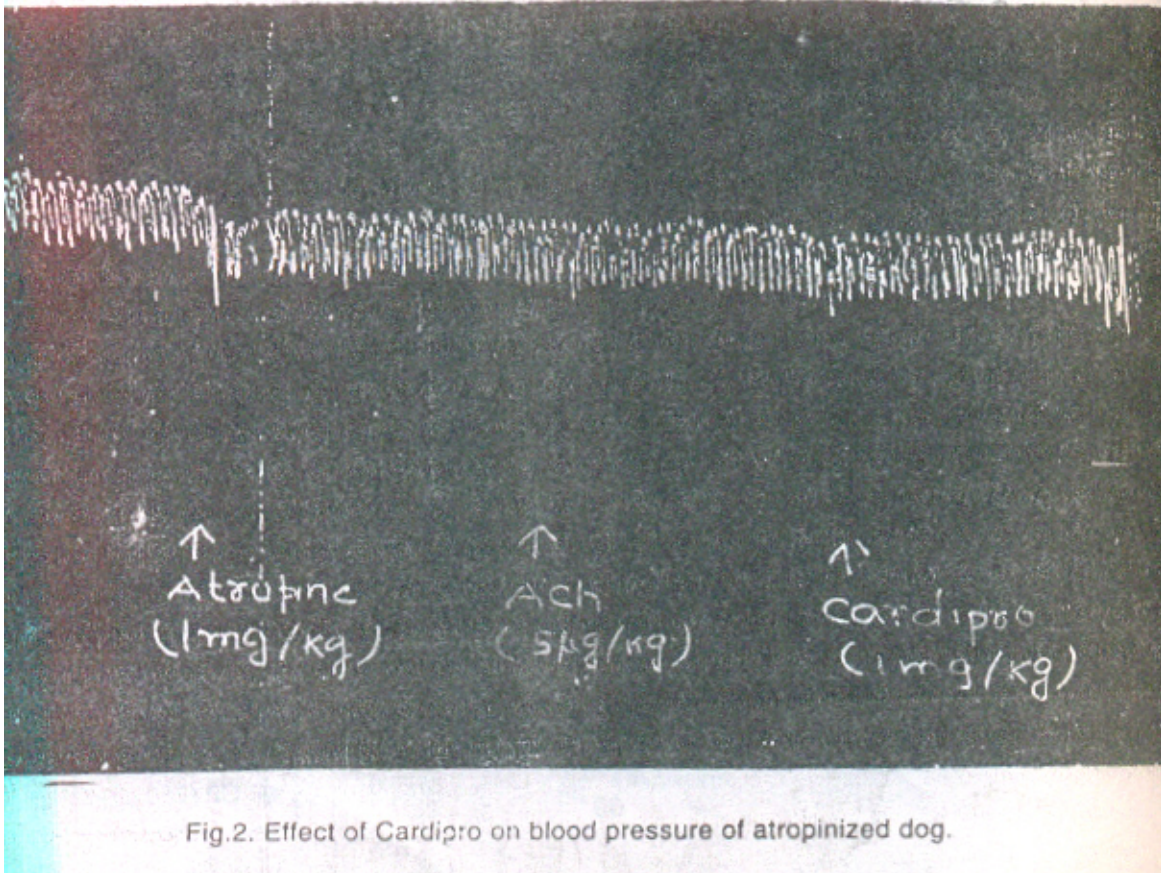
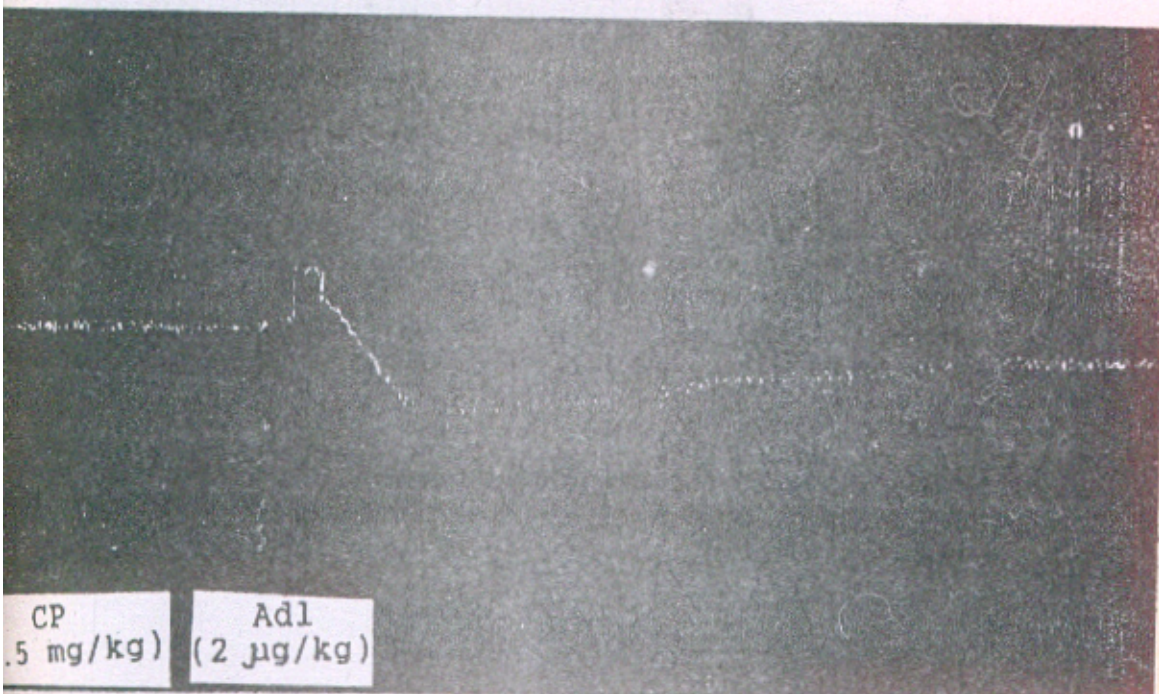
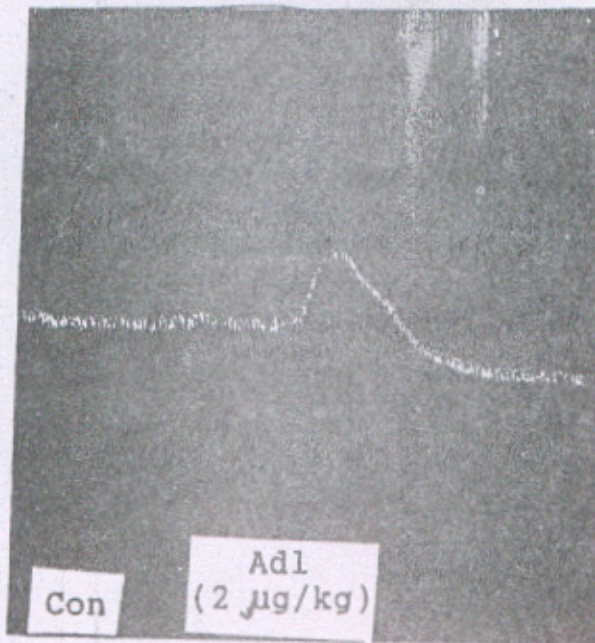


Fig.2. Effect of Cardipro on blood pressure of atropinized dog.





3 Effect of Cardipro on blood pressure response of adrenaline in anaesthetized dogs.

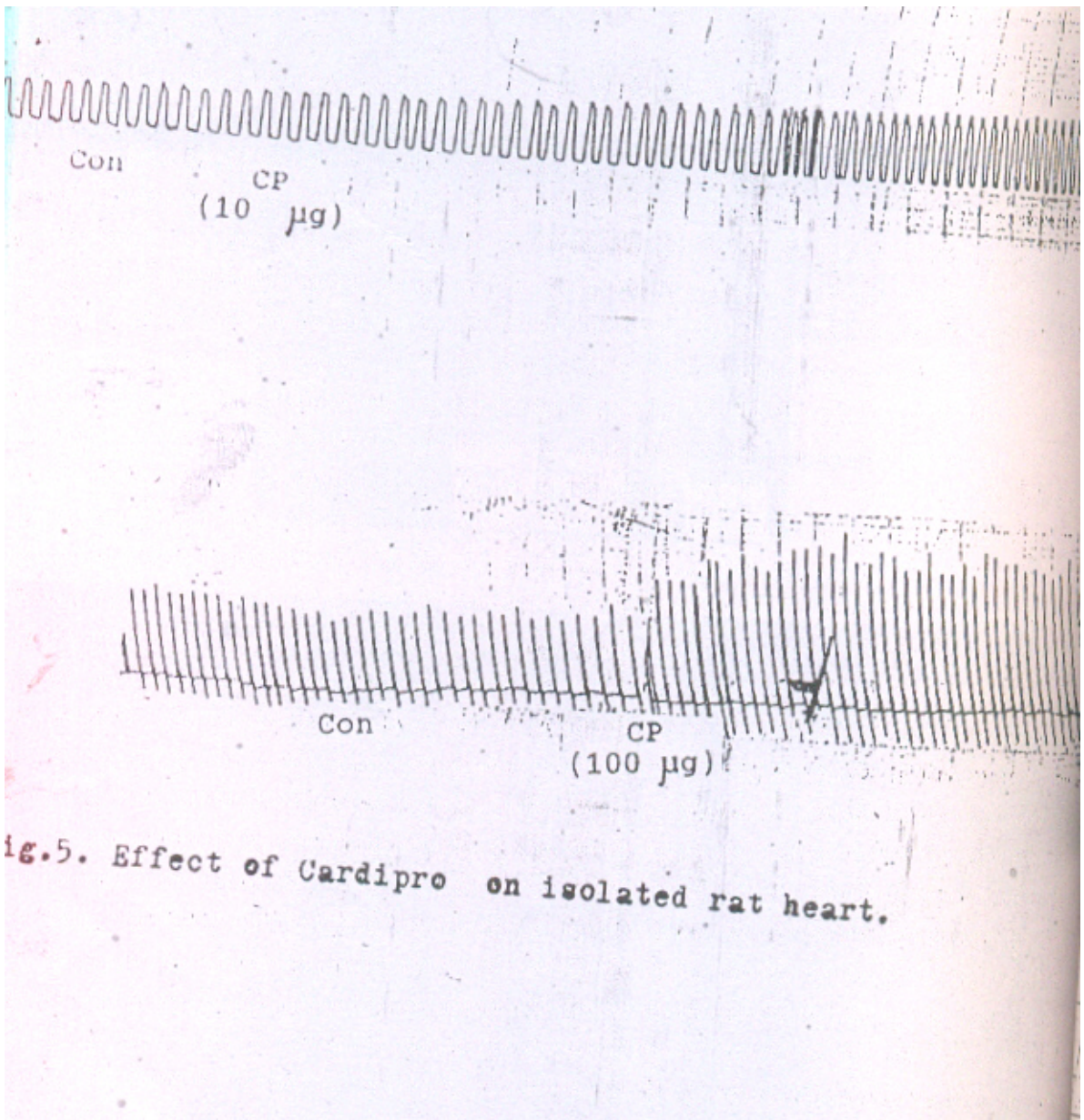


Fig.5. Effect of Cardipro on isolated rat heart.

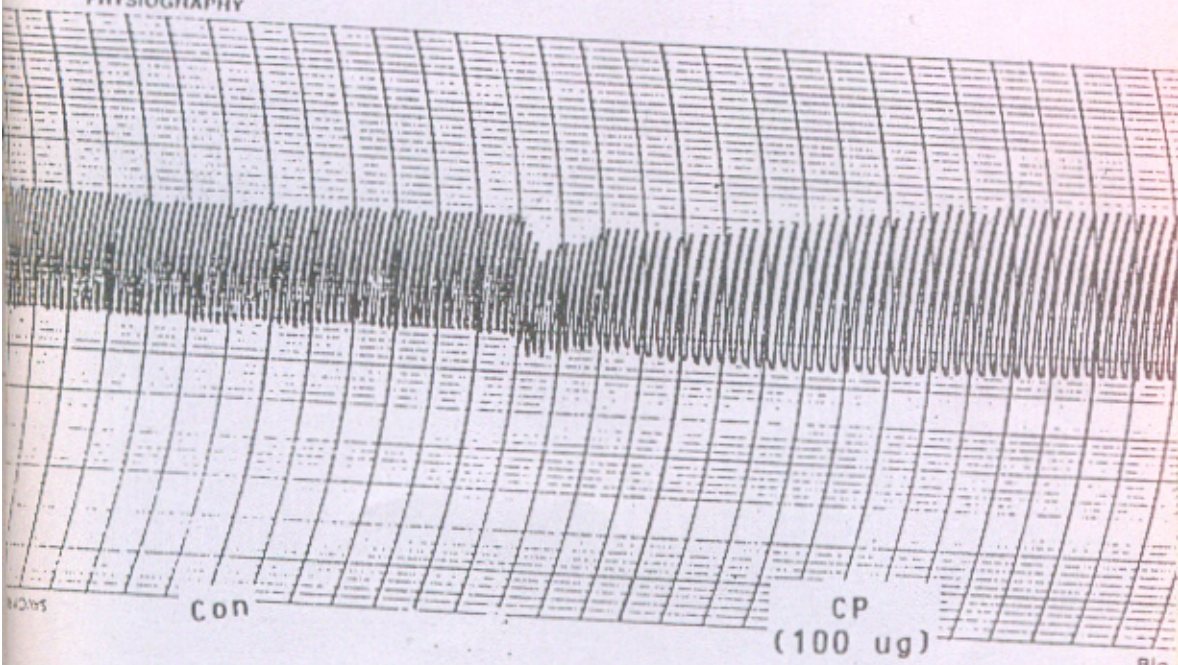




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CP  
(10 µg)

PHYSIOGRAPHY



INDICES

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CP  
(100 ug)

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g.b. Effect of Cardipro on isolated frog heart.



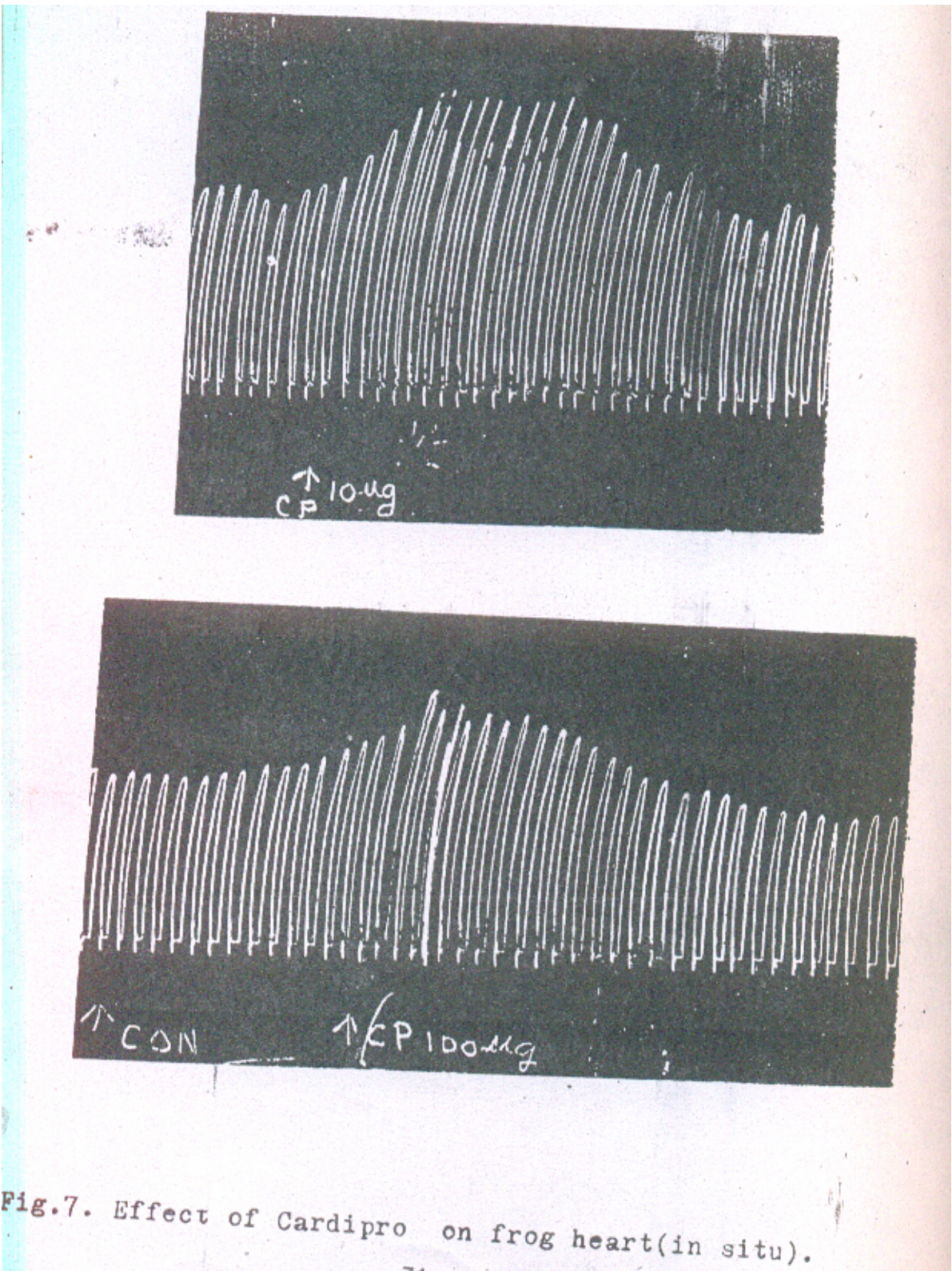


Fig.7. Effect of Cardipro on frog heart(in situ).



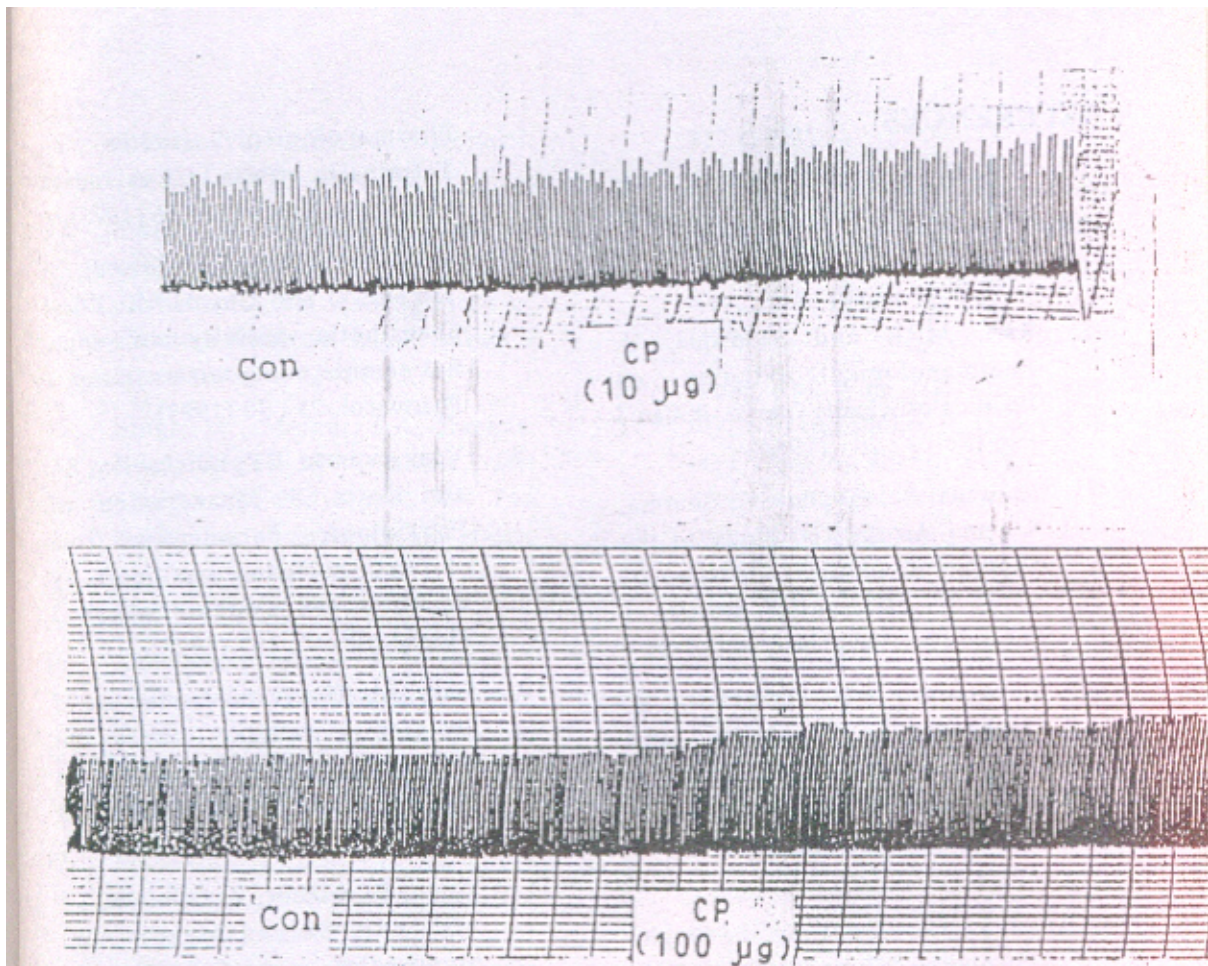


Fig. 9. Effect of Cardipro on isolated rat auricle.

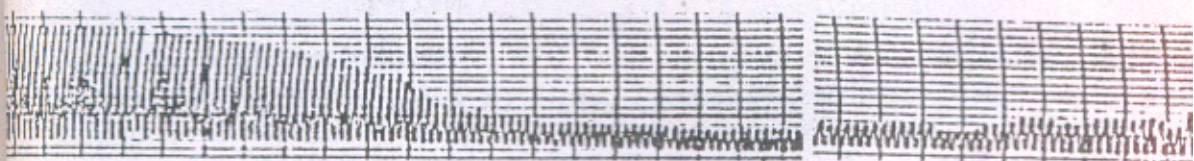


Fig. 8. Effect of Cardipro on isolated hypodynamic frog heart



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