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Full Length Research Paper

Hypotensive effects of 3, 4- dihydroxybenzyaldehyde isolated from the stem bark of *Musanga cecropioides*

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Previous works on the stem bark of *Musanga cecropioides* R. Brown (Moraceae) reported the hypotensive effects of aqueous extract at 10 - 40 mg/kg. This present work was carried out to examine the hypotensive effects of 3, 4-dihydroxybenzaldehyde isolated from the stem bark. Using pentobabitone sodium anaesthetized normotensive rabbits; the effects of the compound on blood pressure were tested at doses of 2.5 - 10 mg/kg. Also, the probable mechanism of action of the compound was examined using atropine, promethazine and the direct effect of the compound on isolated heart. At 2.5 mg/kg, the compound lowered the arterial blood pressure by 12.61 ± 2.45 mmHg. The blood pressure was further lowered by 17.88 ± 0.73 mmHg at 10 mg/kg. The hypotensive effect of the compound was observed to be unaffected by prior administration of either atropine (0.5 mg/kg) or promethazine (0.25 mg/kg). Similar to the mechanism of action of the crude aqueous extract, 3, 4-dihydroxybenzaldehyde induced negative inotropic and chronotropic effects on the heart as it dose-dependently reduced the force and rate of contractions of the heart from 0.5 - 2 mg/ml. The reductions observed at 1 mg/ml were effectively reversed by 10 g/ml adrenaline. However, the effects of 2 mg/ml were not reversed by administration of the adrenaline. The compound seems to be one of the constituents responsible for the hypotensive effect of the aqueous extract of *M. cecropioides* stem bark earlier reported.

Key words: Hypotensive effects, *Musanga cecropioides,* stem bark, 3, 4-dihydroxybenzaldehyde.

INTRODUCTION

High blood pressure is one of the non-communicable diseases affecting many individuals in both developing and developed countries. When not properly controlled, it could lead to life wasting conditions like stroke, and other cardiovascular complications (Lloyd-Jones et al., 2000). There are various synthetic drugs with different mechanisms of actions, which when appropriately taken as prescribed, have remarkable effects in bringing the condition under control. Effective as these drugs may be, some of them have life threatening side effects, and in some cases they are unavailable to many rural dwellers due to precarious drug distribution and manpower re-quired for the administration of such drugs. Researching into medicinal plants with acclaimed effects in reducing blood pressure therefore becomes imperative in other to improve the quality of life of the patients. Such attempts will also help examine and validate or otherwise the scientific rationale underlying the consumption of such herbs by people.

Musanga cecropioides R. Brown (Moraceae) has been reported to be one of the plants used in reducing blood pressure in some parts of tropical Africa (Irvine, 1961). This claim, as well as its uses in inducing labour and hypoglycaemic effects, has been scientifically reported on the leaves and the stem bark (Kamanyi et al., 1991, 1992, 1996; Ayinde et al., 2003, 2006; Ade-neye et al., 2007). From phytochemistry point of view, various parts of the plant has been reported to yield some triterpenoid acids like kalaic, musangic, cecro-pioic acid and a host of others (Lontsi et al., 1987, 1989, 1990, 1991a,b, 1992, 1998a,b). In addition, the leaf was reported to contain orientin, isoorientin, vitexin, pro-cyanidins, chlorogenic acid, and catechin that were observed to inhibit angio-tensin converting enzyme (Lacaille-Dubois et al., 2001)

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while we also reported the isolation of 3, 4-dihydroxy-benzaldehyde from the stem bark (Ayinde et al., 2007). The compound has been reportedly found in *Pinella ternata* (Thunb.) Breit (Araceae) (Nawalade et al., 2003) and Barley tea in which it was demonstrated to be one of the most potent antioxidant components of the plant (Etoh et al., 2004). The probable cardiovascular effect of the compound is yet to be repor -ted in literature. This work was therefore, carried out to examine the probable effect of this natural product on the blood pressure of normotensive rabbits and also the probable mechanisms of action involved.

MATERIALS AND METHODS

Isolation and characterization of 3, 4-dihydroxybenzaldehyde

The stem bark of *M. cecropioides* was collected in Benin City in May 2004 and authenticated by Mr. Kola Odewo at Forest Research Institute of Nigeria (F.R.I.N., Ibadan) where a herbarium specimen No FHI106428 was deposited. After extraction by decoc-tion method using water, the extract was filtered and concentrated using rotary evaporator as reported earlier (Ayinde et al., 2003). Accelerated gradient chromatographic analysis of the ethyl acetate fraction led to the isolation of the compound with R_f values of 0.48 in CH_2Cl_2–CH_3OH (19:1) and 0.82 in $CHCl_3$–CH_3OH (9:1) respec-tively. The melting point was observed to be 152 - 154°C. Using combinations of nuclear magnetic resonance, mass and ultraviolet spectroscopic analyses, and the compound was characterized as 3, 4-dihydroxybenzaldehyde (Ayinde et al., 2007).

Drugs and chemicals

These include promethazine, pentobarbitone sodium (Sigma Chemicals, England),d-glucose, potassium hydrogen phosphate, magnesium sulphate heptahydrate, calcium chloride dihydrate (Merck, England), atropine (Indus Pharma, India), sodium chloride (BDH Chemicals, England), sodium hydrogen carbonate (T.G.I. Ltd, India), potassium chloride (Cambian Chemicals, England).

Animals

Male matured rabbits were purchased and maintained in the animal house of the Department of Pharmacology and Toxicology with standard animal pellets (Livestock feeds, Benin City, Nigeria) and drinking water *ad libitum* until required. Approval for use of the animals was obtained from the Faculty of Pharmacy Ethical Committee on the use of Animals for Experiments.

Effect of 3, 4-dihydroxybenzaldehyde on the blood pressure

Male rabbits weighing 1.8-2.6 kg were used to test the effects of 3, 4-dihydroxybenzaldehyde on blood pressure. Each animal was anaesthetised with pentobarbitone sodium (40 mg/kg i.p.). After cannulating the ear vein for drug administration, the carotid artery was cannulated and connected via a Bentley Physiological pressure transducer to two-channel Ugo Basile recorder (Gemini, 7070) for recording blood pressure and heart rate. The drug was administered intravenously at doses of 2.5, 5, and 10 mg/kg. A waiting period of 10 min was allowed before the administration of the next dose. The mean ± SEM in the fall in mean arterial blood pressure due to each of the doses was obtained from three repetitions.

Experiments on the probable mechanisms of action of the 3, 4-dihydroxybenzaldehyde

Atropine (0.5 mg/kg) was administered into the animal through intravenous route. About 5 min after, 2.5 mg/kg of the 3, 4-dihydroxybenzaldehyde was administered. Similar procedure was followed using promethazine (0.25 mg/kg) (Ayinde et al., 2003).

In order to examine the effects of the compound on isolated rabbit heart, adult rabbits were sacrificed, and the hearts were immediately harvested and placed in dish containing Kreb's physiological solution composed of (mM) -: NaCl 119.0, $NaHCO_3$ 25.0, d-glucose 11.0, KH_2PO_4 1.2, KCl 5.0, $MgSO_4.7H_2O$ 1.2, CaCl. $2H_2O$ 2.6. They were mounted on Langendorff's apparatus connected to a transducer and channel recorder. The hearts were perfused with the physiological solution maintained at 37°C and aerated with 95% O_2 and 5% CO_2. The set-up was allowed to equilibrate for 20 min before the administration of the compound. The effects of the compound on the isolated heart were tested at 0.5, 1.0 and 2.0 mg/ml. The rate of heart contractions was mea-sured by the number of contractions per minute as indicated on the channel recorder, while the heart contraction force was obtained by comparing the effects of the extract in relation to the deflection produced by a tension of 500 mg set on the transducer before administration of the extract. The experiment was carried out in triplicates.

Statistical analysis

All data were expressed as mean ± SEM (standard error of mean) and n represents the number of animals used. Where applicable, the data were compared using one way analysis of variance (ANOVA), Graph pad Instant ^{R} version 2.05a software (UK). The level of significance was from $P < 0.05$.

RESULTS AND DISCUSSION

The 3, 4-dihydroxybenzaldehyde obtained from *M. cecropioides* was observed to affect both the diastolic and systolic blood pressures. The compound exhibited relatively dose dependent reductions in the mean arterial blood pressure of the rabbits used. At 2.5 mg/kg, the compound reduced the mean arterial blood pressure by 12.61 ± 2.45 mmHg and this was further reduced by 17.88 ± 0.73 mmHg at 10 mg/kg (Figure 1) . Although there was no significant difference in the reductions produced by 2.5 and 5 mg/kg, however the effects of 2.5 and 10 mg/kg were significantly different ($P < 0.05$).

Prior administration of atropine (0.5 mg/kg) or prome-thazine (0.25 mg/kg) appeared not to have any effect on the blood pressure reduction activity of the com-pound as the 2.5 mg/kg of the compound showed similar effect observed in the absence of either the atro- pine or prome-thazine (Figure 2). Between 0.5 - 2 mg/ml, the compound produced significant dose related nega-tive inotropic and chronotropic effects on the isolated rabbit hearts. At 0.5 mg/ml, both the force and rate of heart contractions were significantly reduced compared with control levels. The inhibitory effects of the compound at 1 mg/ml were higher

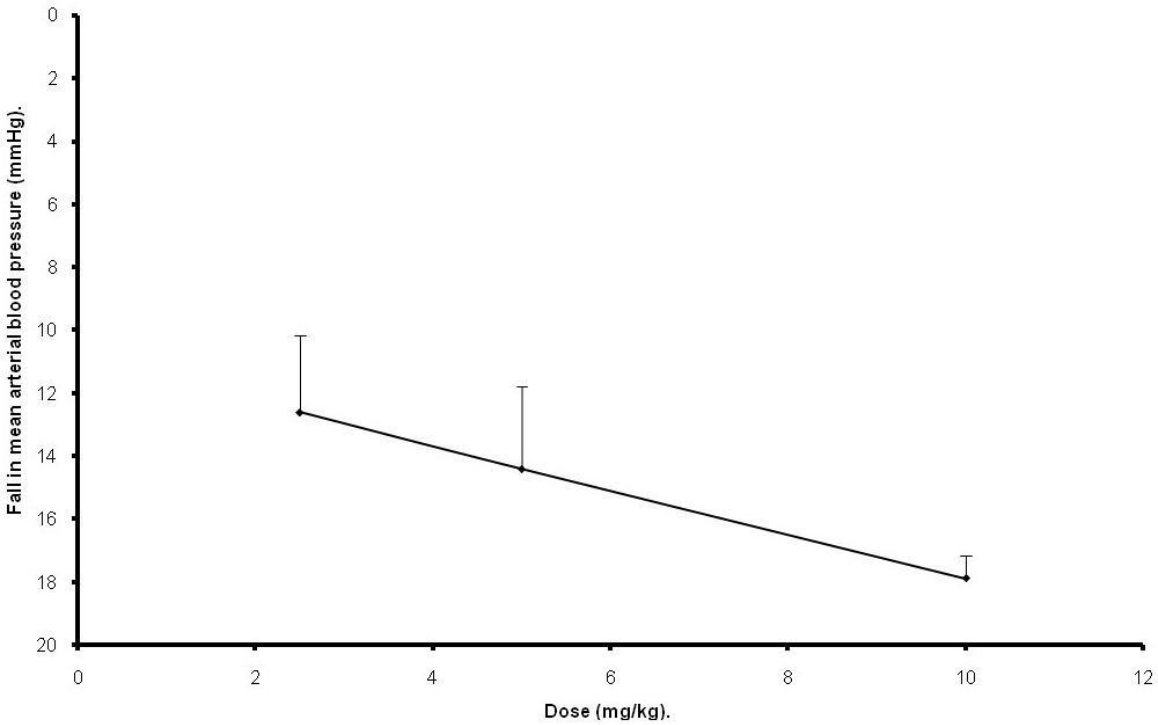

Figure 1. Hypotensive effects of 3, 4-dihydroxybenzaldehyde isolated from the stem bark of *M. cecropioides*. The compound dose dependently reduced blood pressure of the rabbits (n = 3).

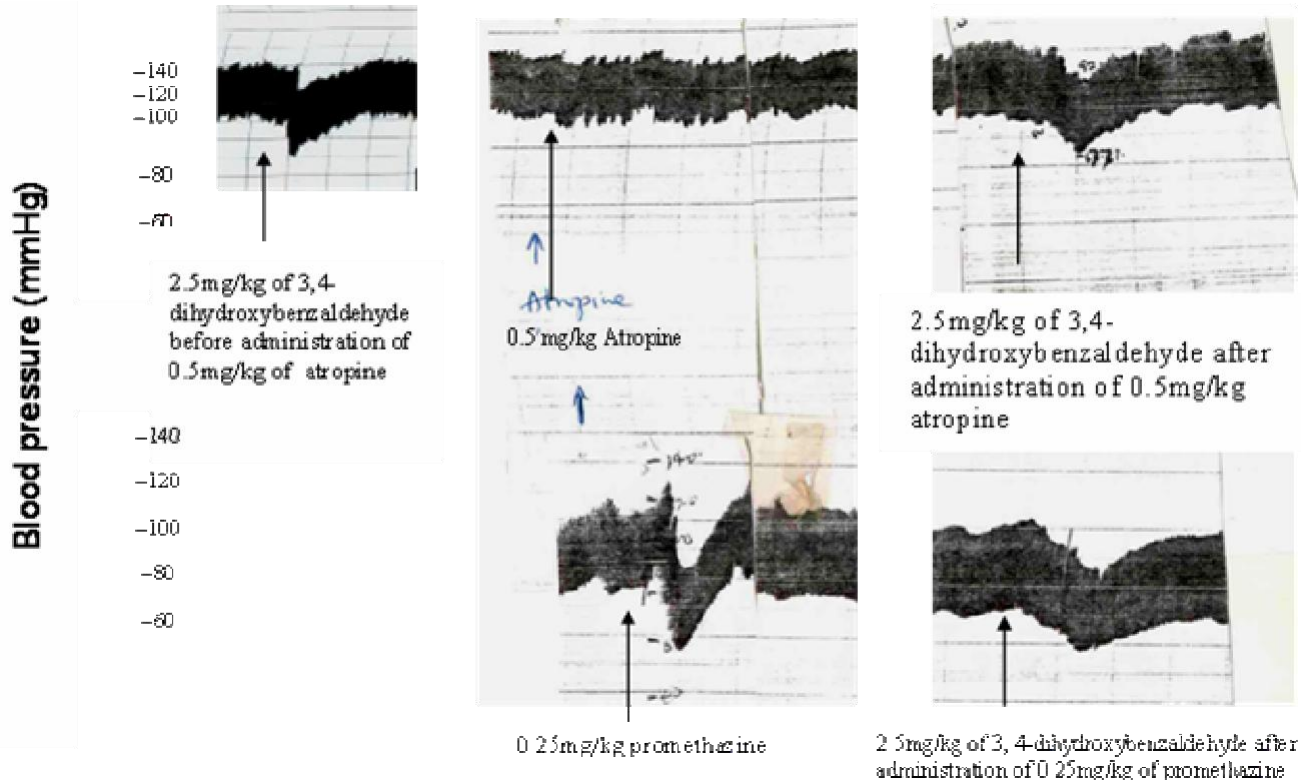

Figure 2. The effects of prior administration of either 0.5 mg/kg atropine (upper part) or 0.25 mg/kg promethazine (lower part) on the blood lowering effects of the 3, 4-dihydroxybenzaldehyde. Neither of the two drugs attenuated the effects of the compound on the blood pressure.

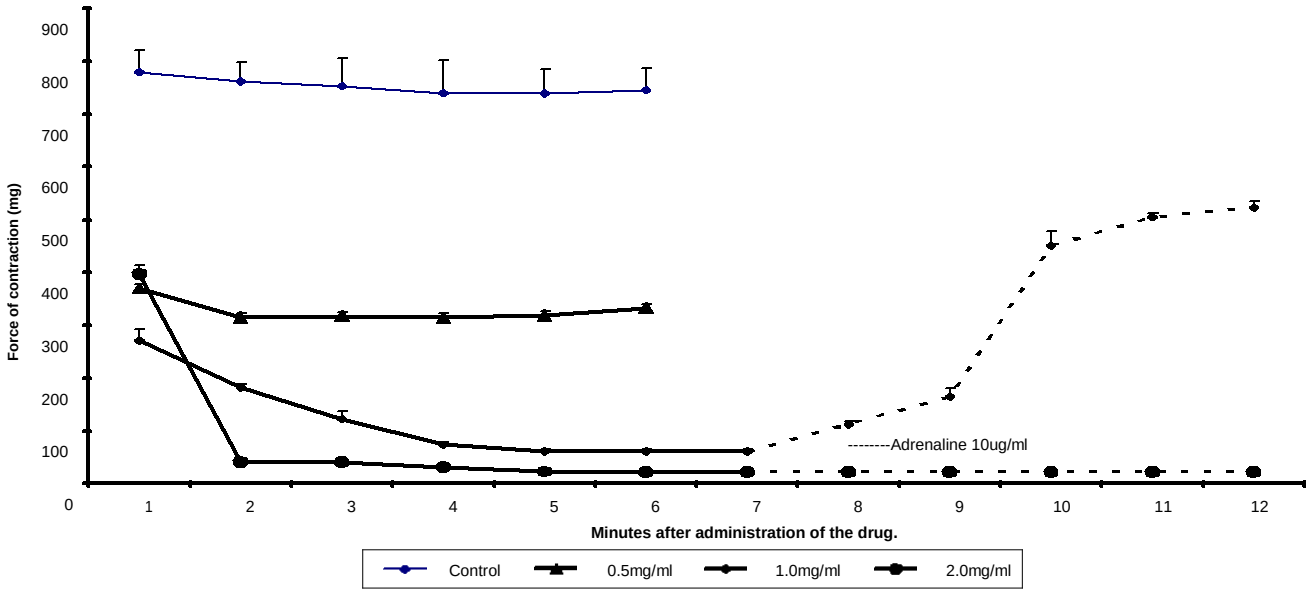

Figure 3. Effect 3, 4-dihydroxybenzaldehyde isolated from the stem bark of *M. cecropioides* on the force of contractions of isolated rabbit heart. It remarkably reduced the force of contractions of the heart at 1 and 2 mg/ml .The inhibitory effect of 1mg/ml was reversed by Adrenaline 10 µg/ml (-------) while the effect of 2mg/ml was irreversible (n = 3).

than those of 0.5 mg/ml while 2 mg/ml exhibited the highest reductions in both the force and rate of contractions. At 1 mg/ml, the inhibitory effects of the compound were effectively reversed by adrenaline 10 g/ml. The reversible effects of adrenaline were more pronounced on the heart rate and even higher than the control levels. However, at 2 mg/ml, the adrenaline showed no effect (Figures 2 - 4).

The hypotensive effects of the aqueous extract of the stem bark of *M. cecropioides* were earlier reported. The 3, 4-dihydroxybenzaldehyde reported to be isolated from the stem bark has been established in this work to possess transient but significant dose-related hypotensive properties. The compound was observed to be more active than the crude aqueous extract earlier reported (Ayinde et al., 2003) as it showed activity at a dose of 2.5 mg/kg contrary to the aqueous extract which slightly reduced blood pressure at a dose of 10 mg/kg as reported earlier.

Useful drugs for controlling elevated blood pressure act through various mechanisms. There are those that act by inhibiting angiotensin-converting enzymes, while some have direct effect on the blood vessels by causing remarkable dilatation. Also, there are some hypotensive drugs that have direct effect on the myocardium (Sun et al., 2002). As we reported earlier for this plant, the mechanisms of action of this compound were found to be similar to that of the crude aqueous extract. Like the aqueous extract, the compound's effect on the blood pressure is unaffected by the presence of either atropine or promethazine. This suggests that the activity of the extract did not provoke the release of histamines neither does it stimulate the muscarinic receptors in the vessels endothelium or the heart. Also, the compound exerts more potent negative inotropic and chronotropic effects on the heart than the crude aqueous extract as reported earlier. This suggests the probable application of the extract and the compound in cases of tachycardia or even arrhythmias. It is possible that the compound reduces the influx of calcium into the myocardiac muscles which consequently affects the rate and force of the heart contraction. Adrenaline is a known vasoconstrictor that produces positive inotropic effects on the heart by stimulating adrenoceptors to increase peripheral vascular resistance, reversing peripheral vasodilation. Simple and polyphenolic compounds obtained from some medicinal have been reported to exert remarkable hypotensive. While gallotannin obtained from *Paeonia lactifolia* was reported to be responsible for the blood lowering effect of the plant through its endothelium- dependent vasodilatory effect (Goto et al., 1996), the hypotensive effect of *Dioclea grandiflora* was attributed to dioclein (5, 2'5'-trihydroxy-6, 7-dimethoxyflavone) - which reportedly reduced mean arterial blood pressure via vasodilation in rats by blocking K^+ channels and inducing membrane hyperpolarization (Cortes et al., 2001). Also, Kang et al. (2003) observed that butein (3, 4, 2'4'-tetrahy-droxychalcone) showed hypotensive effects through endothelium – dependent vasodilation as well as by showing dose-dependent inhibition of angiotensin converting enzyme.

The 3, 4-dihydroxybenzaldehde isolated from the stem bark of *M. cecropioides* has been established in this work to possess hypotensive effect unhindered by the presence of atropine or promethazine but exerts its effect by

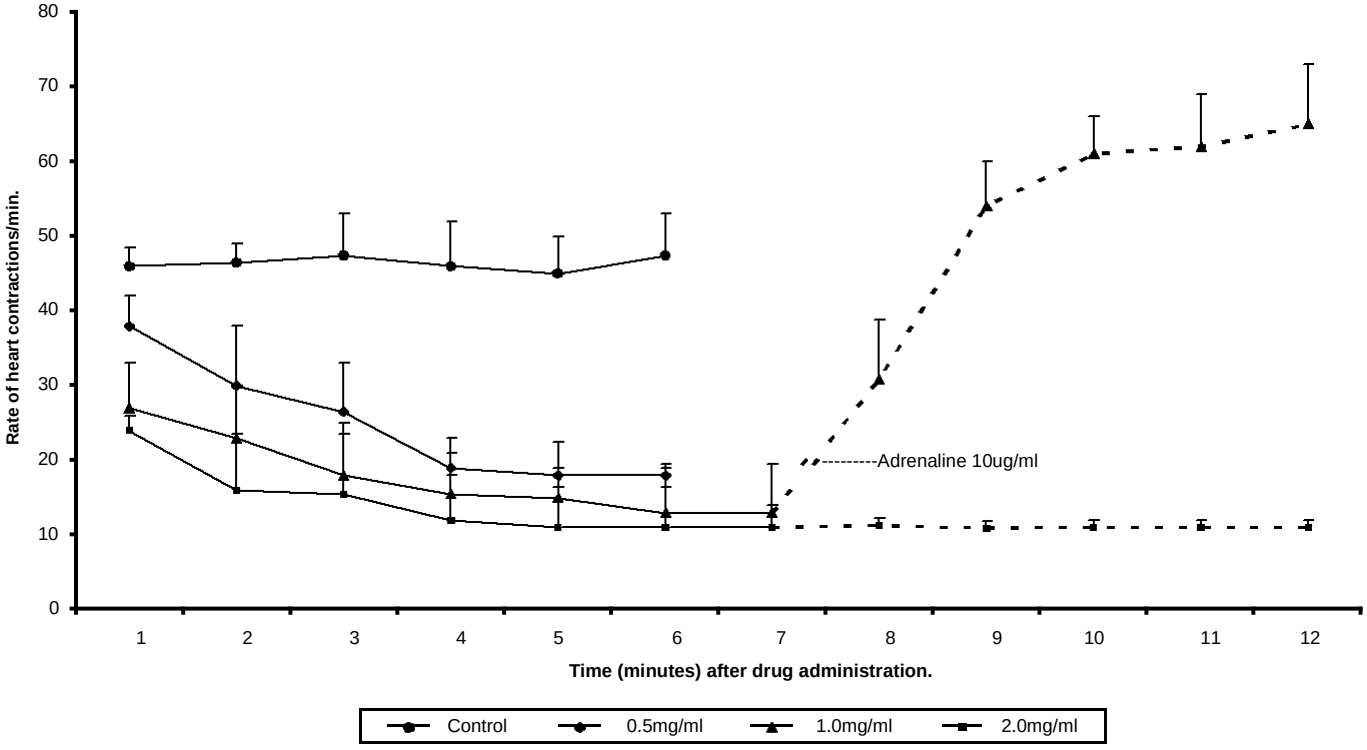

Figure 4. Inhibitory effect of 3, 4-dihydroxybenzaldehyde isolated from the stem bark of *M. cecropioides* on the frequency of contraction of isolated rabbit heart. At 1 and 2 mg/ml it remarkably reduced the rate of contraction of the heart. The inhibitory effect of the sample at 1mg/ml was reversed by 10 µg/ml Adrenaline (----------). At 2 mg/ml the effect became irreversible (n = 3).

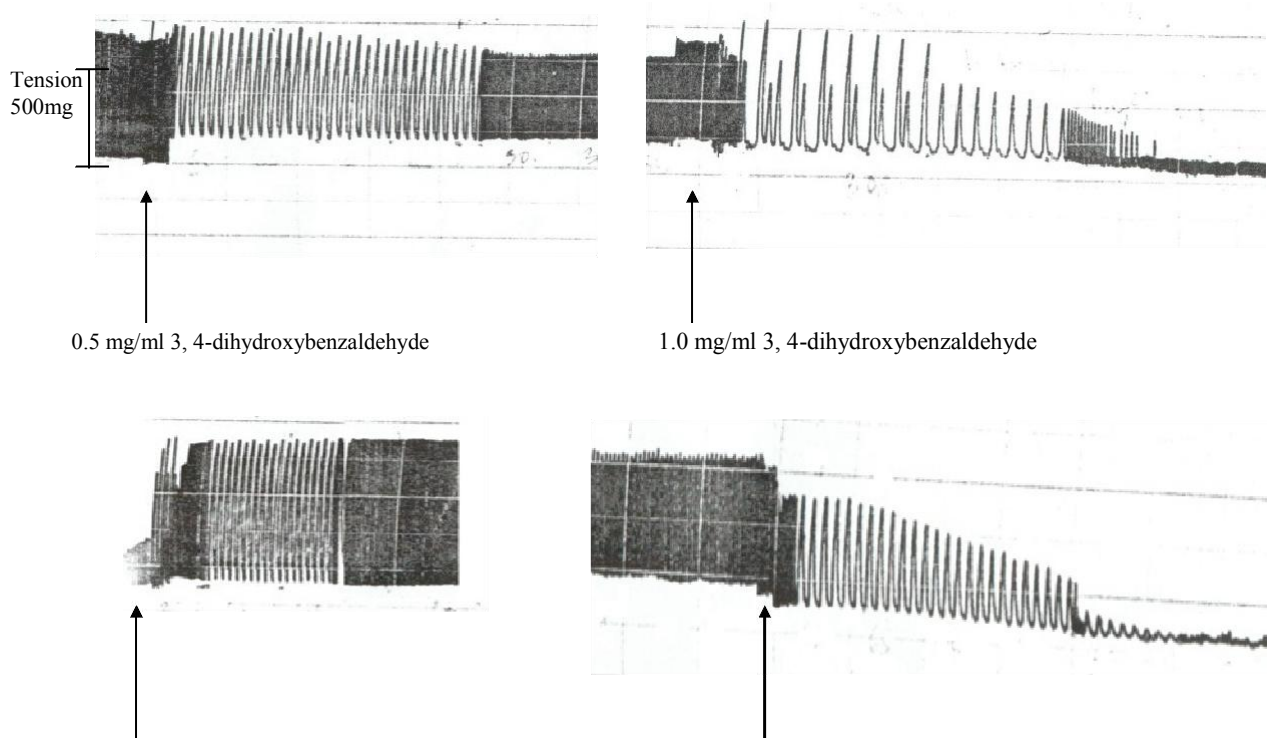

Figure 5. Sample tracings of the effects of 3, 4-dihydroxybenzaldehyde on isolated rabbit heart.

inducing negative introtropic and chronotropic activities on the heart.

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