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Research Article

Vascular Smooth Muscle Relaxation Effects Of The Extract Of The Aerial Parts Of Euphorbia Hirta Linn (Euphorbiaceae) In Wistar Rats

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ABSTRACT

Euphorbia hirta is a small shrub distributed in many countries including China, Malaysia, Australia and many African countries. It belongs to the family Euphorbiaceae. Its effect on vascular reactivity was investigated in healthy wistar albino rats (200-250kg) by the administration of the aqueous extract at doses of 0.625mg/kg – 20mg/kg, to the thoracic aorta in an organ bath containing the physiological salt solution (Krebs solution). The effect of the graded doses of the extract was also evaluated when the thoracic aorta was precontracted with 80Mm potassium chloride and phenylephrine, in the presence of ethylene glycol acetic acid and in its absence. The aqueous extract of Euphorbia hirta caused a significant dose dependent relaxation of the vascular smooth muscle of the thoracic aorta, with the highest at 20mg/kg. The extract was also able to relax the pre-contracted thoracic aorta with 80Mm KCl and phenylephrine in a concentration dependent manner. From the result obtained in this study. It can be concluded that the aqueous extract of Euphorbia hirta contains some constituents which may effective in relaxing blood vessels, and thus has potentials to be in used in the management of elevated blood pressure.

INTRODUCTION

Medicinal plants are those plants that are frequently utilized in treating and preventing particular ailments and diseases that are widely thought to be harmful to humans (Schulz et al., 2001). These plants can either be considered "wild plant species," which are those that grow naturally in plant populations in natural or semi-natural environments and can survive on their own, or they can be considered "domestic plant species," which are cultivated through human activities like selection or breeding and depend on human management for their survival (Calixto ,2000). Long before mankind discovered the existence of microbes, the idea that certain plants had healing potential, indeed, that they contained what we would currently characterize as antimicrobial principles, was well accepted. Since antiquity,

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man has used plants to treat common infectious diseases and some of these traditional medicines are still included as part of the habitual treatment of various maladies (Rios and Recio 2005) Medicinal plants are known to contain some complex chemical constituents responsible for their medical actions, these plants have been used in the development of some orthodox drugs. Over 70,000 plant species are classified as medicinal plants, and because of their chemical constituents, they are used as starting points for conventional drugs (kumar et al.,2010). The phytoconstituents found in medicinal plants are highly abundant and can be converted using cutting-edge technology into brand-new and beneficial medications. Nigeria is home to a wide variety of medicinal plants, and research has determined the efficacy of these plants. Numerous plants have been discovered to contain a variety of antibacterial properties, including guava (Psidium guajava), ginger (Zingiber officinale), neem (Azadirachta indica), and moringa (Moringa oleifera). Alkaloids, polyphenols, terpenes, glycosides, and other phytoconstituents having potential pharmacological effects are found in Nigerian plants, according to research on them. Additional research on these substances will aid in the creation of new generations of therapeutic medicinal agents (Nwinyi et al.,2020) Euphorbia hirta has the characteristic of allomorphic pistillate flowers and fruits (WHO,1991). These are annual, bushy, soft-woody small herb with a thin brownish gray bark, leaves palmately or serrated, lobed flowers are in terminally arranged male flowers on the upper half of the inflorescence and the pistillate at the basal half, fruits globose, dehiscent, it is green and covered with fleshy prickles, seed oblong with smooth, hard, mottle crustaceous testa with a white caruncle at the top enclosing oily endosperm (Lewin et al.,2007). Euphorbia hirta has a worldwide distribution, and its common names include asthma weed and milk weed. Its local name in West Bengal and Bangladesh is "Boro Keruie". It is distributed throughout the temperate or tropical parts of India, Asia, Australia, and Africa, often found in lowland, paddy fields, gardens, waste places, and in the roadsides. They prefer dry and humid condition, from sea-level up to 2200 m altitude. It is native to Central America (Gosh et al.2018). It is a weed considered as beneficial for its diverse application in traditional medicine system. Phytopharmacological investigations showed that its bioactive components possessed various pharmacological properties like anti-inflammatory, antimicrobial, anti-diarrheal, sedative, analgesic, anti-pyretic, anti-oxidant, antiasthmatic, anti-tumor, larvicidal, diuretic, etc (Lanhers et al.,1991). It is extensively used traditionally to cure and prevent gastro-intestinal disorders, afflictions of mucous membranes, and respiratory system disorders ((Shanghai Sci-tech Press, 1986).

Taxonomy of Euphorbia hirta

The botanical classification of Euphorbia hirta is as follows

Kingdom:
Plantae
Phylum:
Magnoliophyta
Class:
Angiospermae
Order:
Malpigiales
Family:
Euphorbiaceae
Genus:
Euphorbia
Species:
hirta
Binomial name :
Euphorbia hirta Linn.
Common name:

Asthma plant, Dove milk, Garden spurge, Hairy spurge, Pillpod sandmat, Pillpod spurge, Red Euphorbia, Snakeweed, Sneezeweed.

Synonym:

E. pilulifera Linn. Chamaesyce pilulifera Linn (Nadkarni, 1976)

Statement of Problem

Hypertension is a deadly disease condition, and its effect amongst Nigerians is enormous. Previously thought to be a condition associated only with the elderly, recent studies has shown that it affects children and young adults too. Due to the side effects associated with conventional anti-hypertensive drugs, the search for herbal drugs that can be used to manage blood pressure has been on the rise, hence this research.

The thoracic aorta

The portion of the descending aorta that is located inside the thorax is known as the thoracic aorta (Latin: aorta thoracica, pars thoracica aortae). The abdominal aorta extends past the posterior mediastinum and is made up of the expanded portion of the aortic arch. The thoracic aorta divides into branches that enter the diaphragm, pericardium, lungs, and other crucial tissues. As a continuation of the aortic arch, the thoracic aorta begins at the base of the fourth thoracic vertebra

OBJECTIVES OF THE STUDY

1. To determine the possibility of the effectiveness of Euphorbia hirta in the management of hypertension
2. To determine the vasodilatory effect of the aqueous extract of Euphorbia hirta on phenylephrine, potassium chloride, in the presence and absence of EGTA induced vasoconstriction.

AIM

The aim of this study is to determine the vasodilatory effect of aqueous extract of Euphorbia hirta plant on denuded thoracic rat aorta and predict the possible mechanism of action.

MATERIALS AND METHODS

Chemicals.

- Aqueous extract of Euphorbia hirta
- Phenylephrine
- High potassium (K+) Potassium chloride solution.
- Ethylene glycol tetracetic acid (EGTA)
- In making the physiological salt solution (Krebs solution) the following chemicals and their respective weights were used;
- Sodium Chloride
- Sodium hydrogen bicarbonate
- D-glucose
- Potassium hydrogen tetraoxosulphate(vi)
- Potassium chloride
- Magnesium tetraoxosulphate(iv)pentahydrate
- Calcium chloride.
- Organ bath
- Recorder

Collection and extraction of the plant

The whole plant of Euphorbia hirta was collected around the premises of University of Benin, between the month of September and October 2021. Identification was done by Late Mr Sonny Nweke of the department of Pharmacognosy, Faculty of Pharmacy, University of Benin and authenticated by Dr Akinibosun of the Department of Plant Biology and Biotechnology, University of Benin. The plant was air-dried for two weeks, and in an electric oven at 40ºc for 60minutes to remove residual moisture content. The leaves and flowers were separated from the stalks and pulverized with an electric milling machine. 120g of the powdered leaf was extracted exhaustively with distilled water using Soxhlet apparatus at 100ºc. 250mg of the extract was dissolved in 5ml of distilled water to obtain 50mg/ml stock solution, from which necessary dilutions were made during experimental procedures.

Animals

Healthy albino rats of both genders, with weights ranging from 200- 250kg were gotten from the animal House of the department of pharmacology and toxicology, University of Benin, where the animals have been fed, and reared at optimum temperature, and had access to clean water. An ethical approval was sent to relevant authorities before any of the animal was sacrificed, in adherence to the laws laid out by the animal ethics committee, University of Benin, Benin City.

Procedure of experiment

The recorder was calibrated to fit the experiment, with a basal tension of 1g. A rat was sacrificed via cervical dislocation, the abdomen was opened with the aid of a sterile scissors to extract the thoracic aorta. A sufficient length of the thoracic aorta was cut out and transferred to the petri dish, which was half filled with the physiological salt solution, to keep it alive. While in the Petri dish, the aorta was cleared off fats that were attached to it, using a sterile scissors, care was taken not to cut the walls of the thoracic aorta, to prevent an altered result. From the denuded thoracic aorta, about 3mm - 5mm was cut out and mounted in an 8.5ml organ bath containing the physiological salt solution. The organ bath was maintained at a temperature of 37oC, with a constant supply of oxygen at optimum pressure. The excised tissue was transferred to the organ bath which already had the aerated physiological salt solution, and properly fixed to the tissue holder. A basal tension of 1g was applied to the tissue and allowed to equilibrate for 60 minutes. During this period of equilibration, the physiological salt solution was changed every 15 minutes. Then at the 60th minute, the vasoconstrictor agent was added. This procedure was carried out for each laboratory protocol.

Phenylephrine vs aqueous extract of euphorbia extract

1M of Phenylephrine stock solution was provided, it was then diluted to 10-7 , 10-6 ,10-5 , 10-4 , 10-3 , 10-2 , and 10-1 molar concentration. The Aqueous extract solution of Euphorbia hirta was diluted to give 0.3125mg/mL, 0.625 mg/mL, 1.25mg/mL, 2.5mg/mL, 5mg/mL, 10mg/mL, and 20mg/mL, using serial dilution methods. After equilibration, 4mL of 10-5 Molar solution of Phenylephrine was administered into the organ bath which had the tissue and physiological salt solution, and a contact time of 5 minutes was allowed. Cumulative doses of 10-4 , 10-3 , 10-2 were administered thereafter, all concentration had a contact time of 5 minutes each. The tissue was allowed to equilibrate to the new environment for 30 minutes. After which, 25µL of 0.3125mg/mL of the Aqueous extract of Euphorbia hirta was administered, a contact time of 5 minutes was allowed. Followed by 50µL of the same Concentration, also with a contact time of 5 minutes. The same was repeated for 125µL, 250µL, and 500µL of the same Concentration. After 5 minutes, the same volume of the extract with the same contact time was repeated for 0.625mg/mL of the extract, the same procedure was repeated for 1.25mg/mL, 2.5mg/mL, 5mg/mL, 10mg/mL, and 20mg/mL. The result was observed and documented.

80mM KCl vs aqueous extract of Euphorbia hirta

80mM of KCl was made in the laboratory, also the Aqueous extract solution of Euphorbia hirta was diluted to give 0.3125mg/mL, 0.625 mg/mL, 1.25mg/mL, 2.5mg/mL, 5mg/mL, 10mg/mL, and 20mg/mL, using serial dilution methods. 5ml of 80mM KCl solution as administered into the organ bath, that already had the fixed tissue in the physiological salt solution. A contact time of 30 minutes was allowed. Then 25ul 0.3125mg/mL, of Aqueous extract of Euphorbia hirta was administered, a contact time of 5 minutes was allowed. Followed by 50µL of the same concentration, also with a contact time of 5 minutes. The same was repeated for 125µL, 250µL, and 500µL, of the same Concentration.

After 5 minutes, the same volume of the extract with the same contact time was repeated for 0.625mg/mL of the extract, the same procedure was repeated for 1.25mg/mL, 2.5mg/mL, 5mg/mL, 10mg/mL, and 20mg/mL. The result was observed and documented.

80mM kcl vs aqueous extract of Euphorbia hirta in calcium free physiological salt solution

Correct amounts of the salts except calcium chloride, were weighed and transferred into a 1L conical flask, and dissolved with distilled water, and made up to 1 litre. 80mM of KCl was made, The Aqueous extract solution of Euphorbia hirta was diluted to give 0.3125mg/mL, 0.625 mg/mL, 1.25mg/mL, 2.5mg/mL, 5mg/mL, 10mg/mL, and 20mg/mL using serial dilution methods. The tissue was allowed to equilibrate in Calcium free physiological solution in the organ bath for 1 hour. Then 5mL, of 10M EGTA was administered, a contact time of 30 minutes was allowed. The tissue was then depolarized with 5mL 80mM KCl, this was allowed till a plateau was seen on the recorder. Then it as washed with the calcium free Physiological salt solution, and allowed to equilibrate for 30 minutes, then 25µL of 0.3125mg/ml of Aqueous extract of Euphorbia hirta was administered, a contact time of 5 minutes was allowed. Followed by 50µL of the same Concentration, also with a contact time of 5 minutes. The same was repeated for 125µL ,250µL, and 500µL of the same Concentration. After 5 minutes, the same volume of the extract with the same contact time was repeated for 0.625mg/mL of the extract, the same procedure was repeated for 1.25mg/mL, 2.5mg/mL, 5mg/mL, 10mg/mL, and 20mg/mL. The result was observed and documented.

1M Phenylephrine vs aqueous extract of euphorbia hirta in calcium free physiological salt solution

Correct amounts of the salts except from calcium chloride, were weighed and transferred into a 1L conical flask, and dissolved with distilled water, and made up to 1 litre. 5ml of 1M EGTA was measured with a micropipette and administered into the bath containing the excised tissue, leaving it to have a contact time of 30 minutes. 1M of Phenylephrine stock solution was provided, it is then diluted to 10-7 , 10-6 ,10 -5, 10-4, 10-3, 10-2, and 10-1, molar concentration. After the contact time between the tissue and EGTA had elapsed, 25µL, 50µL, 125µL, 250µL, and 500µL, of each concentration of phenylephrine was administered cumulatively, each had a contact time of 4 minutes each. The result was observed and documented.

RESULTS

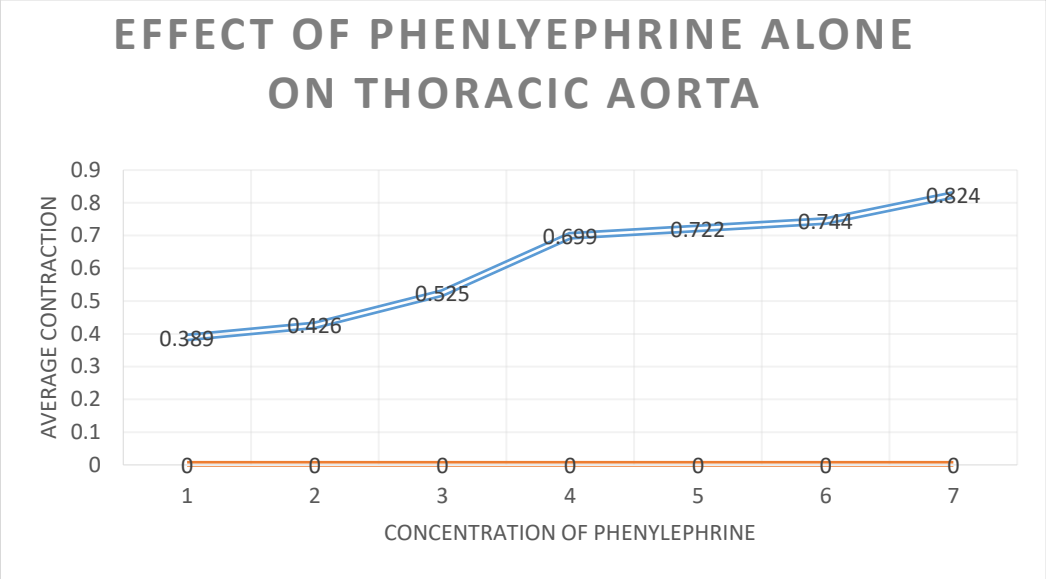

Fig 1. Concentration dependent effect of 1M Phenylephrine on cleared thoracic aorta

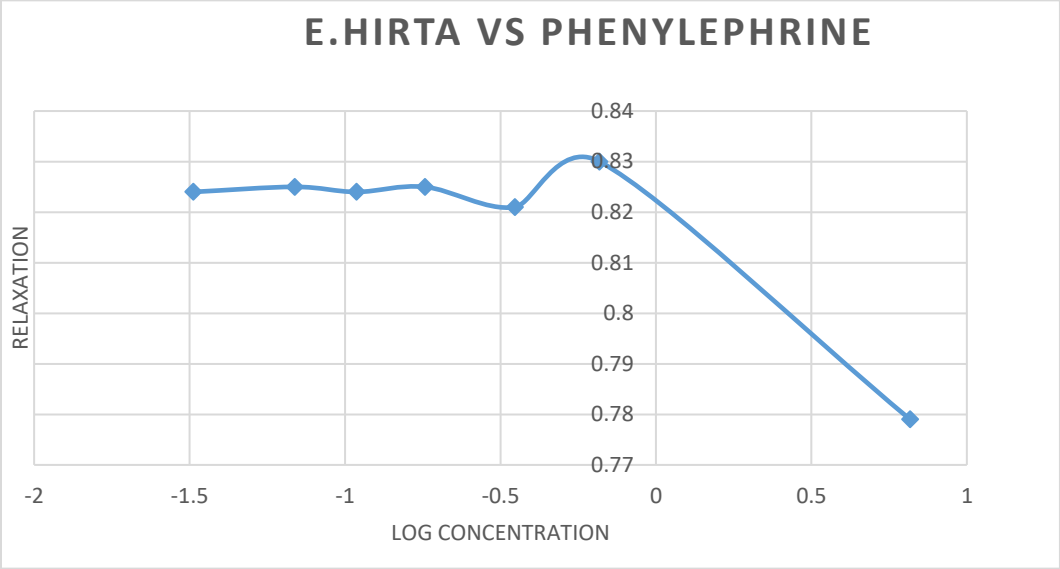

Fig 2 Effect of Various concentration of Euphorbia hirta extract on Phenylephrine contracted thoracic aorta.

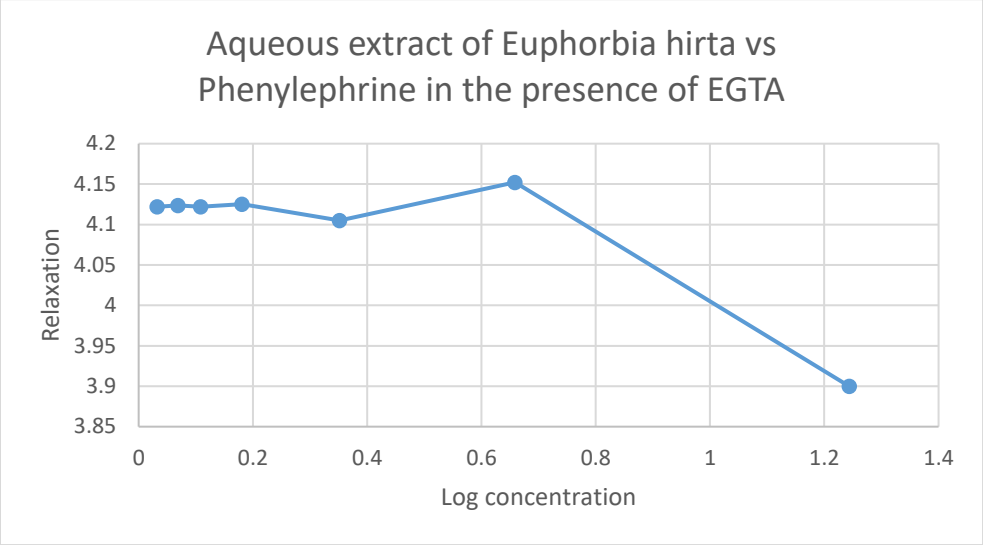

Fig 3 Effect of Various concentration of Euphorbia hirta extract on Phenylephrine contracted thoracic aorta in Calcium free physiological salt solution due to 1M EGTA.

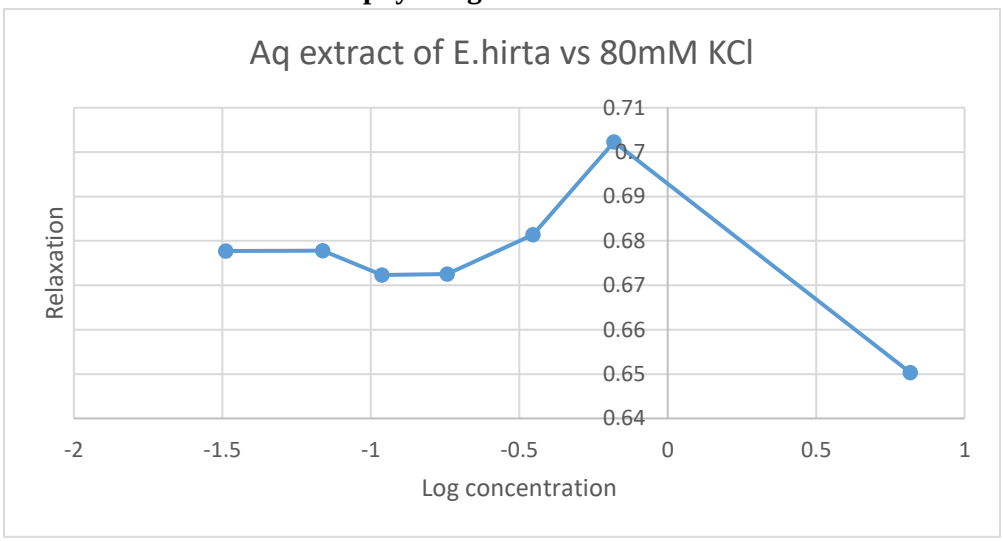

Fig 4 Effect of Various concentration of Euphorbia hirta extract on 80mM Potassium Chloride precontracted thoracic aorta.

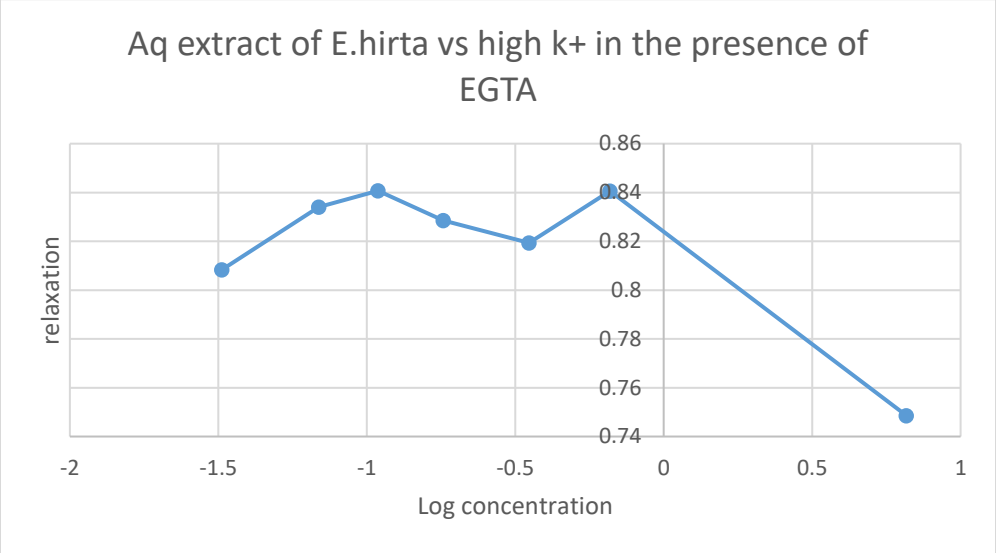

Fig 5. Effect of Various concentration of Euphorbia hirta extract on 80mM Potassium chloride precontracted thoracic aorta in Calcium free physiological salt solution due to 1M EGTA

DISCUSSION

Hypertension is a product of cardiac output and total peripheral resistance, an increase in any of this factor can lead to hypertension. In order to determine its vasodilatory property and possible mechanism of action, the aqueous extract of E. hirta was evaluated for effect on vascular reactivity in rat thoracic aorta, in the presence of PE and 80 mM KCl, and also in the absence calcium ions chelated by EGTA with the contractile agents (PE and KCl) present. The aqueous extract of Euphorbia hirta caused a dose-dependent decrease in vascular contraction induced by Phenylephrine and high K+. Phenylephrine is an α adrenergic agonist, it causes contraction of arteries by binding and activating α-1 receptors. Activation of this receptor, results in the activation of Phospholipase C, which then hydrolyzes phosphoinositol-1,4- bisphoshate in inositol-1,4,5-triphosphate(IP3) and 1,2-diacylglycol, IP3 increases the opening of calcium channels which leads to the influx of extracellular calcium into the cell,increasing intracellular calcium concentration, this then leads to contraction of the vessel (Biazi GR et al.,2018). High k+ (80Mm) is a potent vasoconstrictor, the influx high k+ causes depolarization of the cell membrane, this then leads to contraction of the blood vessel. Ethylene glycol tetracetic acid is a carbon chelator, it binds avidly to calcium, preventing its influx into the cell to cause contraction, this was what caused the initial relaxation seen. After the administration of phenylephrine, we can see that there was an increase in contraction from 10-7 to 10-1, confirming the fact that phenylephrine is a contractile agent. The same thing was seen when high k+ was administered, there was an increase in the contraction of the thoracic aorta, until a plateau was formed. Upon administration of EGTA, there was an initial relaxation of the thoracic aorta seen, but after the contractile agents were administered (Phenylephrine and high k+), a gradual increase in contraction of the thoracic aorta was seen. This is because EGTA was able to chelate all extracellularly available calcium ions, which led to a feedback response, which is the release of calcium ions from the sarcoplasmic reticulum. This feedback response was caused by the contractile agents used. The aqueous extract of Euphorbia hirta, was able to cause a dose dependent reduction in the contraction of the thoracic aorta induced by phenylephrine. Relaxation began at 5mg/kg but the maximum

relaxation was seen at 20mg/kg of the extract. Aqueous E.hirta was also able to reduce vasoconstriction caused by high k+, it inhibited further depolarization of the cell, the relaxation was seen at 20mg/kg. In the presence of EGTA, the extract was also able to cause relaxation, this means that it was able to inhibit further release of calcium from its calcium stores in the sarcoplasmic reticulum. The values where gotten from fixed points gotten from the chart developed by the recorder connected to the organ bath, these values were plotted against each other and the conclusions were properly interpreted.

CONCLUSION

The aqueous extract of Euphorbia hirta has vasorelaxatory properties, being able to reduce vasoconstriction caused by Phenylephrine and High k+ (80Mm) , in the presence and absence of EGTA. The probable mechanism of action is that it inhibits the influx of calcium from its channels, thus causing relaxation by preventing calcium induced vasoconstriction.

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