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INVESTIGATING THE MECHANISM OF ANTIHYPERTENSIVE ACTIONS OF MEDICINAL PLANTS USING SOME ANIMAL MODELS

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ABSTRACT

As the search for, and development of new drugs continues, drug companies engage in the large-scale pharmacological screening of medicinal plants. This creates the need to elucidate the mechanism of action of medicinal plants found to possess biological activity as a means of deriving their full therapeutic potential. This research was carried out to investigate the mechanism of the antihypertensive action of *Vernonia amygdalina*, *Ocimum gratissimum*, and *Pterocarpus erinaceus* using animal models. The dried 70% ethanolic extracts of the plants were prepared at varying concentrations ranging from 0.4 mg/mL to 50 mg/mL. These extracts were administered at varying doses alone and in the presence of selected antagonists like prazocin in anesthetized cat in-vivo and to rabbit jejunum and spontaneously beating guinea pig right atrium. Adrenaline and atropine were used as control drugs.The effects of these plants extracts were demonstrated on the Finkleman preparation and they were found to induce relaxation of the rabbit jejunum. They also reduced both the rate and force of contraction of spontaneously beating guinea pig's right atrium. The cardiovascular effects of the extracts were investigated on cat blood pressure. The effect of atropine tested in the presence of *V. amygdalina* and *O. gratissimum* showed a change in the pattern of induced fall in blood pressure but does block the fall in blood pressure induced by the extracts. While the exact mechanism of the antihypertensive action of these extracts has not been fully determined, the result of this research work proposes that the mechanism could either be blocking calcium channels or have direct activity on lowering blood pressure. It is therefore recommended that further studies be conducted on the extracts to better understand the mechanism of antihypertensive actions of these plants.

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INTRODUCTION

The emphasis on the use of medicinal plants had hitherto been placed on the treatment rather than the prevention of diseases [1]. However, there exists in literature considerable reports in recent times on research work on the use of medicinal plants and their constituents in disease prevention and health promotion [2]. The use of medicinal

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plants for the treatment of diseases dates back to the history of human life, and the information about these plants has long been transmitted gradually from generation to generation [3]. In Nigeria for instance, a meta-analysis survey presents the therapeutic benefits of medicinal plants [4].

Vernonia amygdalina Del (Asteraceae) is a perennial shrub that is widely distributed in tropical parts of Africa [5]. Aqueous extract of *Vernonia. amygdalina* was investigated for its cardiovascular effect in normotensive Sprague-dawley rats. The administration of the extract caused a bi-phasic alteration of blood pressure [6,7]. *Ocimum gratissimum* (O.g) at the dose of 100 and 200 mg/kg improved blood pressure and toxic processes in cobalt chloride-induced cardiorenal dysfunction in rats [8]. *O. gratissimum* is a small aromatic perennial herb grown for its essential oil in its leaves and stem. *Pterocarpus erinaceous* Poir (P.E) is a medium-sized, generally deciduous tree. The phytochemical screening shows the presence of alkaloids, flavonoids, glycosides, phlobatannins, saponins, steroids, and tannins. The plant was investigated to have antihypertensive actions [9].

There are many herbal plants employed in the management of hypertension globally [10]. Some of these plants are used as vegetables or spices in foods. The way some of these plants act to elicit their antihypertensive effects have been studied [11]. However, little or nothing is known about the mechanism of the antihypertensive actions of *Vernonia amygdalina, Ocimum gratissimum, and Pterocarpus erinaceous.*

There are several drug options for the treatment and management of hypertension with known mechanisms of actions. However, the uses of these drugs are not efficient enough to cure hypertension [12]. This made the approach in deriving potent therapeutic agents from medicinal plants to gain more attention [12].

In Nigeria, the use of herbal medicine alone or alongside prescription drugs for disease management is quite common [13]. Somehow, this has been limited to herbal practitioners. Understanding the mechanism of action of these substances will not only encourage their integration into modern medicine but also, prevent issues resulting from drug interactions.

This study is therefore aimed at evaluating the possible mechanisms of the antihypertensive actions of *V. amygdalina, O. gratissimum, and P. erinaceous* using some animal models in the presence of some antagonists.

MATERIALS AND METHODS

Animals

Two pure breeds of American Shorthair cats with an average weight of 3.1 kg were obtained from a household in Farin Gada, Jos. Two male white albino guinea pigs with an average weight of 850 g were obtained from the animal experimental unit of Pharmacology Department, University of Jos. Two Dutch rabbits with the average weight of 2.3 kg were obtained from Mr. Ada's animal farm in Jos.

All the animals were kept in the animal experiment unit under environmentally controlled temperature of 24 ± 1°C. They were acclimatized at least 72 hours before starting the experiments. The animal experimentation was carried out in an ethically proper way by following the guidelines set aside by the Ethical Committee on Animal Experimental of the University of Jos (Reference: UJ/FPS/F17-00379) and the Ethical guidelines for the use of animals in research [14].

Plants Materials

Leaves and stalks of *Vernonia amygdalina, Ocimum gratissimum, and Pterocarpus erinaceous* were collected from Jos metropolis and authenticated at the Department of Pharmacognosy and Traditional Medicine, Faculty of Pharmaceutical Sciences, University of Jos by Mr. Thomas Yakubu. The voucher numbers issued were UJ/PCG/HSP/95C16, UJ/PCG/HSP/16L06, and UJ/PCG/HSP/16F18 respectively.

Preparation of the Extracts

The plants materials were air-dried and pulverized into powder. The powders were mixed with 70% ethanol. The setups were macerated for 72 hours with regular shaking at room temperature (24 ± 1^{o}C). The samples were filtered using Whatman paper 1 and the filtrates were evaporated to dryness over water bath maintained at 78 ^{o}C.

In vitro Studies on Effect of Extracts on Rabbit Jejunum (Finkleman Preparation)

The Finkleman preparation of the rabbit small intestine [15] was adopted. Two adult male rabbits were sacrificed and their jejunum cut helically into strips of 3 - 4 cm. The tissue was mounted in a 50 ml organ bath containing tyrode solution.

The organ bath, which was connected to a three-channel student physiograph, was bubbled with air and the temperature of the solution containing the tissue was maintained at 37 ^{o}C. The strips of the rabbit jejunum were placed under an initial passive tension and allowed to equilibrate. Adrenaline (0.4 mcg) and acetylcholine (0.4 mcg) respectively were

used to check the viability of the tissues. Then, 0.4 mg, 4 mg, and 40mg concentrations of *V. amygdalina* were used to investigate the effect of the extract on the tissue. The 40 mg concentration was used to check the activity profile of the tissue for 15 minutes. Prazocin (50 mcg) was used in the presence of 0.4 mcg adrenaline to determine the dose of the antagonist to be used. The extract was challenged with the prazocin. The procedures were repeated for the other extracts.

In vitro Studies on Effect of Extracts on the Spontaneously Beating Right Atrium of Guinea Pig

Two adult guinea pigs were sacrificed and the right atria were isolated. The tissue was mounted in an organ bath containing Ringer Locke solution, bubbled with oxygen and the temperature maintained at 37 ^{o}C. The organ was connected to the transducer of a power lab calibrated at the sensitivity of 2 mV and the tissue allowed to rest for 30 minutes. Adrenaline (20 mcg) and acetylcholine (20 mcg) respectively were used to check the responsiveness of the tissue to the drugs. Effect of *V. amygdalina* (20 mg) was determined alone and in the presence of prazocin (20 mcg) on the tissue. The procedure was carried out with the other extracts.

In vivo Studies on Effect of Extracts on Anaesthetized Cat

Two cats were anaesthetized with a 5 ml/kg of 25 % urethane given intravenously. The animals were mounted and prepared for recording of blood pressure. Standard drugs and the extracts were then injected into the femoral vein. Using a cannula, arterial blood pressure was recorded through a pressure transducer and the phasic arterial pressure monitored by a 3-channelphysiograph. Adrenaline and acetylcholine were administered at the dose of 10 mcg and 1 mcg respectively. The extracts at the doses of 10, 40, and 50 mg were administered. A 20 mcg atropine was administered followed by 0.5 ml normal saline and 1 mcg acetylcholine. The extracts at the dose of 50 mg were administered in the presence of 20 mcg atropine. The pattern of administration follows this order; atropine (20 mcg) followed immediately by 0.5 mL normal saline and 50 mg of each extract.

RESULTS

Effect of *V. amygdalina, O. gratissimum and P. erinaceous* on rabbit jejunum

Acetylcholine induced a dose dependent relaxation of rabbit jejunum while adrenaline caused a dose dependent relaxation of rabbit jejunum (Figure 1).

V. amygdalina produced a dose dependent relaxation of the rabbit jejunum. Lower doses of 40 mcg, 400mcg, and 4 mg of the extract reduced both the rate and height of contraction of the tissue but the relaxation was not complete. At the dose of 40 mg, *V. amygdalina* induced total relaxation of the rabbit jejunum (Figure 2). The relaxation persisted even after 15 minute of exposure (Figure 3). *O. gratissimum* at the dose of 40 mg was able to induce complete relaxation of rabbit jejunum. This induced relaxation occurred faster than equivalent dose of *V. amygdalina*. This effect was not blocked by 10 mcg/ml prazocin.

Pterocarpus erinaceous at the dose of 80 mg induced complete relaxation of rabbit jejunum. This activity was delayed in the presence of 10mcg/ml prazocin but prazocin was unable to block it.

Effect of *V. amygdalina and O. gratissimum* on the Spontaneously Beating Right Atrium of Guinea Pig

Adrenaline at the dose of 0.4 mcg/ml caused an increase in both the force of contraction and the rate of contraction of the spontaneous beating of the right atrium of guinea pig (Figure 4), while acetylcholine at the same dose caused a decrease in the activity of the spontaneously beating right atrium of guinea pig (Figure 5).

V. amygdalina and *O. gratissimum* induced a decrease in the force and the rate of contraction of the spontaneously beating right atrium of guinea pig (Figure 6 and 7 and chart 1 respectively).

Effect of *V. amygdalina, O. gratissimum, and P. erinaceous* on Cat Blood Pressure

O. *gratissimum* caused a dose dependent fall in cat blood pressure. It produced an immediate but sustained fall in cat blood pressure. At the dose of 10 mg, it reduced blood pressure by 4 mmHg. At the dose of 40 mg, it reduced cat blood pressure by 13 mmH while at the dose of 50 mg, it induced the fall in blood pressure by 9 mmHg. There was a changed in the pattern of fall in blood pressure in the presence of 50 mcg atropine. Interestingly, atropine was unable to cause a significant change in the induced fall in blood pressure at the dose of 50 mg, as the blood pressure reduction remained 9 mmHg.

V. amygdalina displayed a dose dependent fall in blood pressure of cat. The fall in blood pressure was immediate and sustained. At 10 mg, *V. amygdalina* reduced blood pressure by 10 mmHg.

At 40 and 50 mg, it produced a biphasic action with a transient rise in blood pressure (1 mmHg) and an immediate but sustained reduction in blood pressure. At dose of 40 mg, blood pressure decreased by 13 mmHg. At the dose of 50 mg, blood pressure decreased by 12 mmHg.

P. erinaceous induced a dose dependent fall in cat blood pressure. At the dose of 10 and 40 mg, it caused an immediate but transient fall in blood pressure. At the dose of 50 mg, it produced an initial rise in the blood pressure of 1 mmHg and a sustained fall in blood pressure. 10 mg produced a 3 mmHg reduction in blood pressure; 40 mg produced a 6 mmHg fall in blood pressure while 50 mg caused a fall in blood pressure by 7 mmHg (Figure 8). 50 mcg of atropine was able to block significantly the fall in blood pressure induced by a 50 mg of *P. erinaceous.*

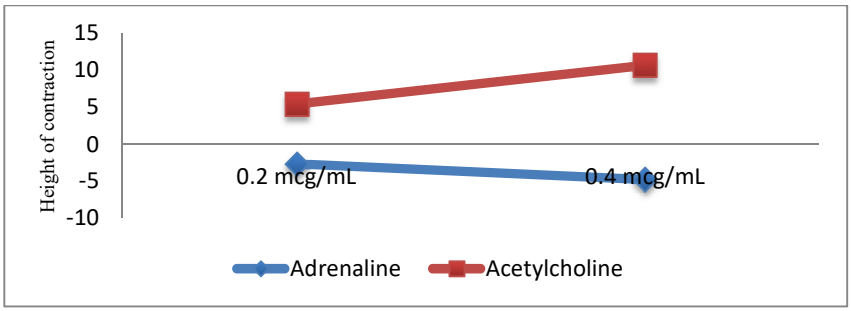

Figure 1. Effect of varying doses (0.2 mcg/mL and 0.4mcg/mL) of adrenaline and acetylcholine on rabbit jejunum.

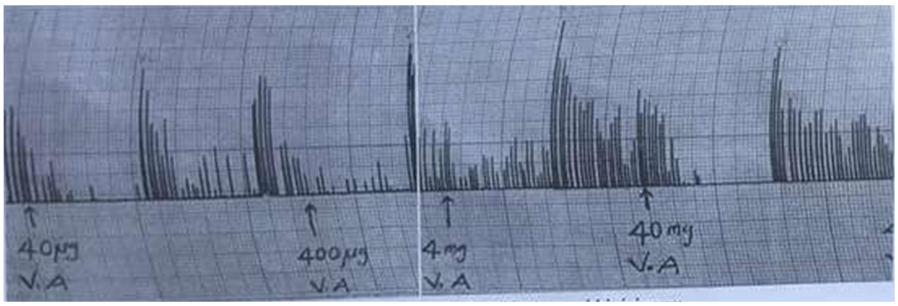

Figure 2. Effect of varying doses of *V. amygdalina* on rabbit jejunum

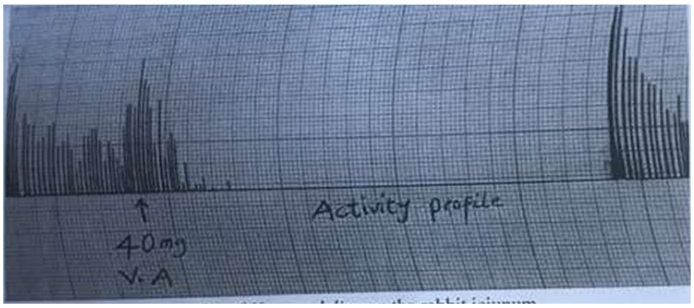

Figure 3. Activity profile of *V. amygdalina* for 15 minutes on rabbit jejunum

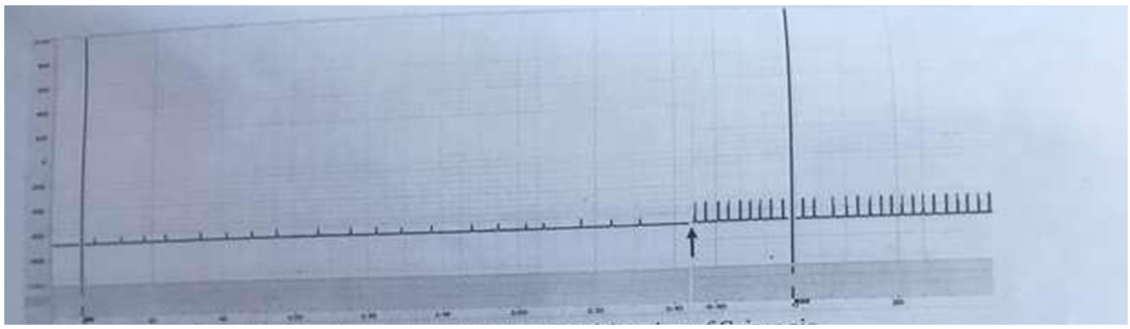

Figure 4. Effect of adrenaline on the spontaneously beating guinea pig right atrium
The rising arrow shows the point of administration of the 20 mcg adrenaline
Vertical axis represents force of contraction while the horizontal axis represent rate of contraction

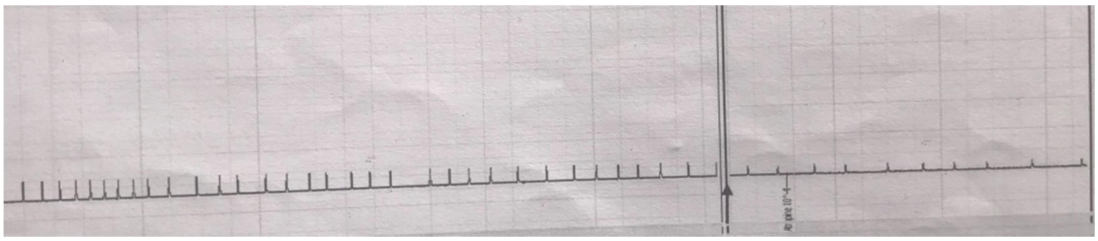

Figure 5. Effect of acetycholine on the spontaneously beating guinea pig right atrium
The rising arrow shows the point of administration of the 20 mcg acetylcholine
Vertical axis represents force of contraction while the horizontal axis represent rate of contraction.

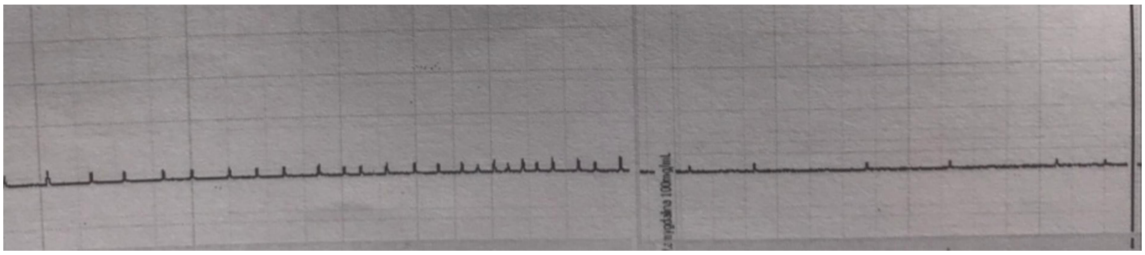

Figure 6. Effect of *V. amygdalina* on the spontaneously beating guinea pig right atrium
The printing on the chart shows the point of administration of the 20 mg V. amygdalina
Vertical axis represents force of contraction while the horizontal axis represent rate of contraction.

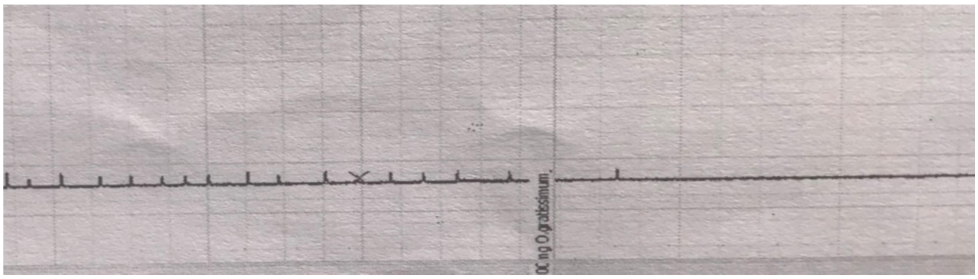

Figure 7. Effect of *O. gratissimum* on the spontaneously beating guinea pig right atrium.
The printing on the chart shows the point of administration of the 20 mg *O. gratissimum*
Vertical axis represents force of contraction while the horizontal axis represent rate of contraction

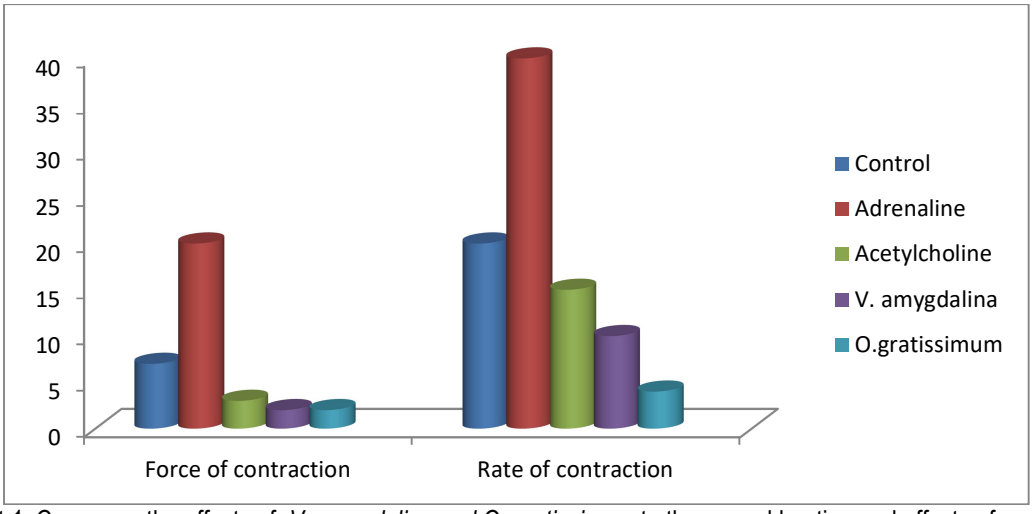

Chart 1. Compares the effects of *V. amygdalina and O. gratissimum* to the normal beating and effects of standard drugs (adrenaline and acetylcholine) on the spontaneuosly beating guniea pig right atrium.
The vertical axis represents length of contraction in millimeter and beats per 6 seconds respectively.

Figure 8. The effect of 10 mg, 40 mg, and 50 mg of *P. erinaceous* on anesthetized cat blood pressure

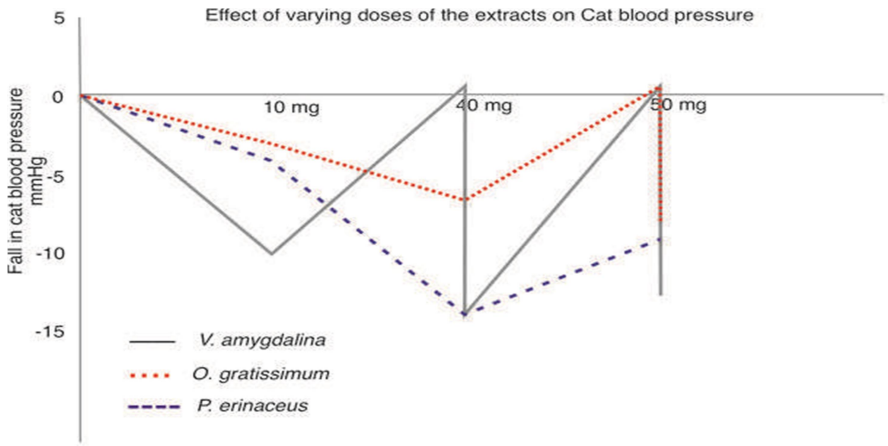

Figure 9. Effect of varying doses of the extracts on anesthetized cat
The zero point represents the baseline blood pressure of the cat

DISCUSSION

The effects of *Vernonia amygalina, Ocimum gratissimum, and Pterocarpus erinaceous* were demonstrated on the Finkleman preparation and they were found to induce relaxation of the rabbit jejunum. Adrenaline induced a dose dependent relaxation of the rabbit jejunum similar to these extracts and it therefore could be suggested that the mechanism of action for these extracts can

either be one or more of the followings; sympathetic agonists, calcium channel blockers e.g. nifedipine and/or a direct vasodilator e.g. hydralazine, organic nitrate. Effect of prazocin (alpha 1 blocker) on induced relaxation of the extracts on this tissue showed that the effects of the extracts were not mediated through this receptor, as prazocin could not block their effects. However, there was a delay in the activity of *P. erinaceous*, which was not clearly understood. Similar result was obtained in 2019 [16], where it was suggested that the relaxation action of the essential oil of *O. gratissimum* is likely to be due to a direct effect on the smooth muscle of the rabbit jejunum rather than an indirect action on neurotransmitter release. Aliyu and Chidi had a similar result [17] and suggested that the extract of *P. erinaceous* interacts with muscarinic receptors on the rabbit jejunum. This could possibly prevent calcium influx through the voltage operated channels by inhibiting the calcium induced calcium release mechanism. This prevents the release of calcium ion from the sarcoplasmic reticulum thereby, closing the sodium and calcium ion channels, action of second messengers like cAMP, or preventing binding of the calcium to calmodulin.

To further investigate and narrow down the mechanism of actions of these extracts, their effects were tested on the spontaneously beating right atrium of guinea pig. *V. amygdalina and O. gratissimum* were found to reduce both rate and force of contraction of the tissue.

Adrenaline as expected induced an increase in contraction (rate and force) of guinea pig heart's activities, which were the opposite effects, produced by the extracts. This can therefore exclude any speculation that sympathetic agonist is one of the possible mechanisms by which the extracts elicit their actions. This narrows down their activity to either calcium channel antagonist and/or direct activity.

The cardiovascular effects of the extracts were investigated on cat blood pressure. The fall in blood pressure observed on the intravenous administration of *V.amygdalina* on cat blood pressure is similar to a 2010 report [18]. There was an initial rise blood pressure and immediate but sustained fall in blood pressure. Intravenous administration of *O. gratissimum* induced a similar sustained and a dose dependent fall in blood pressure of cat [16]. Effect of atropine tested in the presence of *V. amygdalina* and *O. gratissimum* showed a change in the pattern in induced fall in blood pressure but does block the fall in blood pressure induced by the extracts. The profile of fall in blood pressure observed in this study suggests that the extracts may have active principle(s) that behave like acetylcholine.

P. erinaceous at the dose of 10 and 40 mg induced an immediate but transient fall in blood pressure and the pattern of blood pressure reduction was similar to that of acetylcholine. However, at the dose of 50 mg, there was a rapid and slightly sustained fall in blood pressure near the basal (normal) blood pressure, which was significantly blocked by atropine. This may be due to stimulation of muscarinic cholinoceptors on the heart. Since *P. erinaceous* relaxed the rabbit jejunum rather than contraction, it is possible that this effect may be due to some action not related to alpha 1 stimulation.

CONCLUSION

The blood pressure lowering effects of the ethanolic extracts of *V. amygdalina, O. gratissimum, and P. erinaceous* have been demonstrated against standard antagonists using three (3) different animal experimental models. While the exact mechanism of antihypertensive action of these extracts have not been fully determined, the result of this research work proposes that the mechanism could either be blocking calcium channels or have direct activity on lowering blood pressure.

It is therefore recommended that further studies be conducted on the extracts to isolate and characterize the component responsible for blood pressure lowering effect of the plants. Also, additional investigation should be carried out to elucidate the exact mechanism of smooth muscle relaxation and blood pressure lowering action of the extracts.

CONFLICTS OF INTEREST

The authors declared that they have no conflict of interests.

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