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The Effect of ethanol Psidium Guava leaf extract on KCl Induced Contracted Aortic tissues of Guinea Pigs

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Abstract: Hypertension is elevation of systolic blood pressure greater than or equal to 140 mm Hg and/or diastolic blood pressure greater than or equal to 90 mm Hg. It affects 1 billion people and accounts for approximately 7.1 million deaths annually. It is significant risk factor for cardiovascular disorders. Some medicinal plants are traditionally used to treat hypertension. Psidium guava is one of widely cultivated medicinal plant used as traditional medication for diabetes, vomiting, diarrhea and dyslipidemia. The study was designed to identify effect of P. guava leaf extract on hypertension in guinea pigs. The study was conducted on six male guinea pigs to measure the effect of ethanol P. guava leaf extract by using invasive method. The result was analyzed by using paired t-test, independent student t test, one –way ANOVA. Ethanol extract of P. guava leaf showed a significant (p= 0.001) ability to relax isolated aortic tissue of guinea pigs in dose dependent manner, after induction of aortic muscle contraction by high potassium chloride concentrations (80 mM KCl). The effects of P. guava leaf extract in relaxation contracted aortic tissue was may be due to numerous effect of phytochemicals.

Keywords: Aortic Tissue, Blood Pressure, Cardiovascular Diseases, Effect, Extract, Guinea Pigs, Hypertension, P. Guava

1. Introduction

Non-communicable diseases have long duration and generally slow progression. Basically, it can be categorized as cardiovascular diseases (CVDs), cancers, chronic respiratory diseases and diabetes [42]. They have many negative impacts on human health and economy [18]. CVDs are considered as one of major cause of death [42]. Low and middle-income countries bear a large burden of CVDs account 80% of world CVD-related deaths and 87% of disability may be due to accessing fast/ sweet foods that may contribute for different CVDs [15].

Hypertension is elevation of systolic blood pressure (SBP) greater than or equal to 140 mm Hg and/or diastolic blood pressure (DBP) greater than or equal to 90 mm Hg [23]. Increasing in its prevalence leads to dramatic rises in the incidence of CVDs and their consequences [10], [24], and [18]. It is one of the most common global health problems, affecting approximately one billion people and accounts for approximately 7.1 million deaths annually [29]. Different factors such as gender, age, genetic factors, alcohol consumption, dyslipidemia, smoking increases its severity [5], [10]. It can be classified into primary resulted from sedentary lifestyle and diet (accounts for 90-95%) and secondary resulted from chronic kidney disease which accounts for 5-10% [21], [22]. Although mechanisms of primary hypertension are not well known, secondary hypertension can be resulted from defect in arterial sub-endothelial cells; renin-angiotensin-aldosterone system; central nervous system dysfunction, leading to elevated sympathetic activity [13].

Diuretics, angiotensin converting enzyme (ACE) inhibitors, angiotensin receptor blockers (ARBs), calcium channel blockers (CCBs), alpha and beta blockers are drugs

that used in hypertension treatment. All these drugs have their own side effects [36].

Medicinal plants are rich with ingredients that have been used in drug development [15]. According to report by [20], between 70 to 80% of the world's population relies on herbal medicine. Over three-quarters of the world population relies on plants for their health care [14]. About 80% of the Ethiopian populations depend on traditional medicine from plants for their healthcare [3]. About 300 of the traditional medicinal plant species of Ethiopia are frequently mentioned as sources for medicinal value [3], [4]. Blood pressure can be reduced when smooth muscle are relaxed. Potassium channel opening, calcium channel blocking and receptor antagonism are mechanisms that contribute Smooth muscle relaxations [12]. *Annona* leaf extract in normotensive Sprague Dawley rats was reported to lower elevated BP due to presence of essential oils present in the plant exhibiting vasodilator activities [27]. Garlic is another herb that can reduce BP by stimulating nitric oxide production, resulting vasodilatation [26].

Psidium guava (P. guava) is a small medicinal tree. It is native to Mexico, Central America, and northern South America. Its family is Myrtaceae. There are two common varieties of guava: the red (*P. guajava*var. *pomifera*) and the white (*P. guajava*var. *pyrifera*). Its fruits is one of the most popular and rich with nutrition and has been used traditionally as a medicinal plant throughout the world for a number of ailments [1]. It is low in calories and fats but contains numerous vitamins, minerals antioxidants, polyphenolic and flavonoid compounds that may play important role in prevention of CVDs [4], [32]. Potential medicinal effects of *P. guava* fruit, roots, leaves or branches have been described in animal and human studies [19]. Though there are no published studies on medicinal effects of *P. guava* in Ethiopia, *P. guava* is recommended as a traditional medicine for various ailments, including nausea and vomiting, diarrhea, and as an antimicrobial in different parts of the country. Animals and human studies suggested that *P. guava* leaf, root, fruit or branches may be used to treat diabetes and dyslipidemia [2], [8], [14], [26], [33], [35], [37]. Anti-inflammatory effects of *P. guava* have also been described [6], [9], [39], [41]. Another study also suggested that *P. guava* may have strong anti-oxidant properties [30], [40]. Hepato-protective and anti-cancer effects have also been suggested in non-human studies [17], [25], [28], [31]. However, some studies showed negative effects of *P. guava* on spermatogenesis in rats [7]. Its effects may be due to the presence of many different phytochemical compounds such as vitamin C, vitamin A, iron, calcium, manganese, phosphoric, oxalic and malic acids, saponin combined with oleanolic and others [16], [34]. Quercetin, α-pinene, β-pinene, limonene, menthol are compounds from its leaves; polyphenols and resin exist in its bark; gallic acid and tannin are found in its roots; and phenolics and flavonoids are found in its seed and wings [22], [38]. Therefore, this study was designed to examine the effect ethanol *P. guava* leaf extract on relaxation of aortic muscles in guinea pigs.

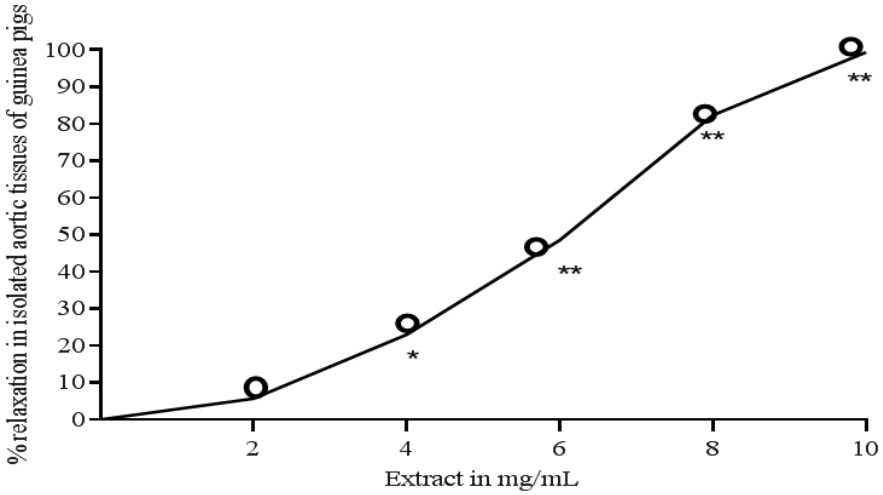
2. Materials and Methods

The study was conducted at Addis Ababa University, School of Medicine, laboratories of Biochemistry, Physiology, pharmacology animal house and Ethiopian Public Health Institute. The study protocol was approved by research and ethical committee of Biochemistry department. The matured fresh leaf of *P. guava* was collected, washed and dried under shaded area. Then it was extracted by 70% of ethanol. Dose for this experiment was determined based on pilot studies, because no pre-existing data was available in the medical literature. Six male guinea pigs weighing 400 - 600 grams were given one week acclimatization period in the pharmacology animal house. They were allowed free access to water and cabbage at controlled ambient temperature of 22 ± 2°C and 50 ± 10% relative humidity, with 12 hour light/12 hour dark cycles. Guinea-pigs were sacrificed stunning and cervical dislocation. Descending thoracic aorta was quickly removed and placed in Krebs-Henseleit solution which was made from NaCl 6.9 g/L, $NaHCO_3$ 2.1 g/L, $CaCl_2$0.36 g/L, KCl0.373 g/L, $KH2PO_4$0.16 g/L, $MgSO_4$0.141 g/L and glucose 2g/L at pH 7.4. Excess fat and connective tissues were trimmed off. Aorta was cut spirally resulting in long strip. From this strip a short strip (2 to 3 cm) was prepared for the experiment. The aortic tissue was kept moist with Krebs-Henseleit solution during the whole procedure and the strip preparation was mounted in a 2.5 ml tissue bath containing Krebs-Henseleit solution, maintained at 37 ^{0}C and continuously bubbled with a mixture of 95% O_2 and 5% CO_2. A resting tension of 1g was applied to the tissue and an equilibrium period of 1hour and 10minutes was allowed to equilibrate before addition of any *P. guava* leaf extract. High K^+ (80 mM KCl) was added to the bath solution to induce sustained contraction of the aortic tissue. During this period fluid was changed at every 15minutes. The *P. guava* leaf extract was later applied to check relaxation of aortic contraction induced with high K^+ (80 mM). Contraction that induced by KCl was accepted as 100%. When the contracted tissues were treated with extract. The relaxation was determined by percent in opposite direction to contraction. A change in isometric tension of the strip was measured via a force displacement transducer (FT- 03) using a grass model 7E polygraphs [11].

3. Result

During the experiment high KCl concentration (K^+ =80mM) was induced to contract guinea pig aortic tissues (100%). Then the contracted aortic tissues were treated with various doses of ethanol leaf extracts of *P. guava* (2, 4, 6, 8 and 10 mg/mL) as shown in (Figure 1). The mean percentage relaxation of guinea pig aorta treated with 2mg/mL of extract was found to be 5.72 ± 3% and it was not significantly relaxed (p=0.536 as compared with the baseline contraction). However, treatment of isolated aortas with increasing doses of leaf extract caused significant (p=0.05, p= 0.001) relaxation of aortic tissues. The mean percentage relaxation

of aortas at higher doses of extract were 22.95±2.5% for 4 mg/mL extract; 49.55± 3.50% for 6 mg/mL extract; 82.35± 4.1% for 8 mg/mL extract; and 99.37± 2.9% for 10 mg /mL extract), (p=0.05 for extract doses between 4mg/mL and 6 mg/mL extract and p=0.001 for 8mg/mL and 10 mg/mL extract). This was dose-dependent, with higher doses of extract causing progressively greater relaxation of aortic muscle than lower doses (Figure 1).



Comparison was done with one way ANOVA, and p <0.05 was considered significant, *= (p=0.05), **= (p =0.001), o= point of treatment.

Figure 1. *Effect of P. guava leaf extracts on contracted aortic tissue of guinea pigs.*

4. Discussion

Hypertension is a common health problem, affecting approximately one billion people globally, and accounts for 7.1 million deaths annually according to the report by [29]. It is major risk factor for cardiovascular diseases. Its prevalence is increasing throughout the world, including Ethiopia and the rest of sub-Saharan Africa, according [19], [24]. Medicinal plants have been used in treating of mainly different types of diseases. Human also prefer them due to having of inadequate supply of drugs, side effects of allopathic medicines, resistance to drugs and high cost treatments [33]. And also might be due to its easiness to access and low costs of plants. According to [14], over three-quarters of the world population relies mainly on plants and plant extracts for health care. Many medical plants' products act synergistically, and they may also act synergistically with synthetic pharmaceuticals [34]. *P. guava* has many traditional medicinal uses in different countries, including Ethiopia.

In this study aortic tissues of guinea pigs were treated with high concentrations (80Mm) of potassium chloride. It was applied to induced maximum contraction (100%) to the aortic tissues of guinea pigs. As reported by [11], potassium chloride at high concentration (>30mM) can induce smooth muscle contractions. This is mainly might be through opening of voltage-dependent Ca^{2+} channels, thus allowing influx of extracellular $Ca^{2+,}$ causing a contractile effect [4].

The contracted guinea pigs aortic tissues by high potassium chloride (K+=80mM) was not significantly (p=0.536) relaxed when aortic tissues were treated with dose of 2 mg/mL of *P. guava* leaf extract as compared to baseline contraction. This indicates only 5.72 ± 3% of contracted aortic tissues were relaxed. This may indicate few contents of active ingredients that is responsible to induce relaxation of aortic tissues. The mean percentage of relaxation of extract treated contracted aortic tissues of guinea pigs with doses of 4 mg/ml and 6mg/ml were 22.95±2.5% and 49.55± 3.50 respectively. Here there was significant (p=0.05) relaxation of contracted aortic tissues of guinea pigs as compared as base line contraction (100%). The mean percentage of relaxation of extract treated contracted aortic tissues of guinea pigs with doses of 8 mg/ml and 10 mg/ml were 82.35± 4.1% and 99.37± 2.9% respectively which indicates the aortic tissues were significantly reduced as compared to the base line contractions. This relaxation is may be due to vasodilation effect of quercetin, lycopene and other active ingredients' of *P. guava* leaf extract. Study done by [21] on efficacy and mechanisms of quercetin in treatment of hypertension, shown vasorelaxation of isolated aortas of rats, by inhibiting the action of ET-1 at the gene transcription level, and promoted the production and action of nitric oxide on endothelium cells. Another report done by [39] showed that *Hibiscus hispidissimus* results reduction in blood pressure by scavenging nitric oxide radical and also inhibition lipid peroxidation.

The relaxation of these aortic tissues may be due to either potassium channel opening or calcium channel blocking or receptor antagonism or synergistically. As reported [12] smooth muscle relaxation can be achieved by various mechanisms such as potassium channel opening, calcium channel blocking and receptor antagonism. So plant chemicals may have role in either of these mechanisms. As explained by [30], *Thymus vulgaris showed capability to block calcium channels that leads to relaxation of smooth muscles.*

As observed in figure 1, the mean percentage relaxation of

contracted aortic tissues treated with doses 2mg/ml, 4mg/ml, 6mg/ml, 8mg/ml and 10mg/ml of ethanol extract of *P. guava* leaf were 5.72 ± 3%, 22.95±2.5%, 49.55± 3.50, 82.35± 4.1% and 99.37± 2.9% respectively shows relaxation of tissue in doses dependent manner. A study reported by [27] on the effect of annona leaf extract on rats' isolated aortas, showed significant relaxation in dose dependent manner.

5. Conclusion

In conclusion hypertension is server risk factor for cardiovascular diseases. In order to reduce this risk factor different types of drugs have been used worldwide. But almost all of them have their own side effects. Many plants are assumed to be medicines for different types of diseases including hypertension. Therefore, this study was designed to examine the effect ethanol *P. guava* leaf extract on relaxation of aortic muscles in guinea pigs. Since the relaxations of smooth muscles reduces blood pressure. The *P. guava* is one of the most common plant used in protecting different types of diseases. The *P. guava* leaf was extracted by 70% ethanol. Aortic tissues of guinea pigs were contracted by inducing high potassium chloride. Different doses of ethanol extract of *P. guava* leaf were applied to contracted tissues of guinea pigs. The *P. guava* leaf causes relaxation of guinea pigs aortic tissues in dose dependent manner.

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