



RESEARCH ARTICLE

**Study of Anti-Hypertension Activity of Aerial Parts of
Passiflora foetida Linn.**

Ranganatha N*, Dr.I.J.Kuppast, Veerashekar T
Department of Pharmacology, National College of Pharmacy, Shimoga, Karnataka.

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*Corresponding author: rangs8988@gmail.com

ABSTRACT

Hypertension is one of the leading causes of disability, mortality, and morbidity along the populace. *Passiflora foetida* Linn. is an Indian medicinal plant which belongs to the family Passifloraceae. The plant has been reported to contain alkaloids and flavonoids. Hence the present study has been designed to evaluate the anti-hypertensive activity of ethanol, petroleum ether and ethyl acetate extracts of *Passiflora foetida*. The method of “up and down” (OECD Guideline No. 425) of CPCSEA was adopted for acute toxicity studies. The anti-hypertensive activity was carried out by measuring systolic and diastolic blood pressure in awaked animals by the non-invasive blood pressure module. Hypertension was induced by administering 8% salt (NaCl) solution for two weeks. Amlodipine (5 mg/kg) was used as a standard drug. In the present study ethyl acetate extract (300mg/ml) of *Passiflora foetida* has been shown significant anti-hypertensive activity. The anti-hypertensive effect produced by ethyl acetate extract of *P. foetida* may be due the activation of NO-dependent vasodilation and also due to the presence of flavonoids in the ethyl acetate extract. Hence it can be concluded that the ethyl acetate extract of *Passiflora foetida* Linn. has the potential to become a good anti-hypertensive drug. Further studies are needed to know the exact mechanism of action.

Key words: *Passiflora foetida* Linn. Aerial parts. Anti-hypertensive. Systolic. Diastolic.

INTRODUCTION

Cardiovascular diseases account for 12 million deaths, annually worldwide. Hypertension is one of the leading causes of disability, mortality, and morbidity along the populace. It is the most common chronic illness among the world faces (Akinkigbe O 2001 and Schutte A et al. 2003). Hypertension is the most common cardiovascular diseases and constitutes a major factor for several cardiovascular pathologies including atherosclerosis, coronary artery disease, and myocardium infarct, renal insufficiency, and stroke and dissecting aneurysm of aorta (Oparil S et al. 1999). Various drugs and regimes have been advocated for the control of hypertension. Many new drugs have been introduced which may demonstrate better efficacy but posses side effects. Recently attention has been focused towards herbal and mineral preparations which are traditionally used as potential therapeutic agents in the prevention and management of cardiovascular diseases (Bhatt J. D et al. 1998). *Passiflora foetida* Linn. is an Indian medicinal plant which belongs to the family Passifloraceae It is an herbaceous climber,

native of Tropical America and found wild in several parts of India (The Wealth of India 1996). It is commonly called as Stinking passion flower in English, Mupparisavalli, Siruppunaikkalli in Tamil, Tellajumiki in Telugu, Kukkitaballi in Kannada and Chadayan, Poochapazham in Malayalam (Narayan Das Prajapati et al. 2003; Khare C.P et al. 2007 and Norman Grainger Bisset et al. 2001). The whole plant is used in the treatment of insomnia and anxiety (Manuchair Ebadi et al. 2007). The decoction of fruit is used for asthma and biliousness. Decoction of leaves and roots is emmenagogue and also used in hysteria. The plant is used for curing itches. The major components present in the plant are Maltol, phytosterols, cyanogenic glycoside, flavonoids and their glycosides (Balasubramaniam A et al. 2010).

MATERIALS AND METHODS

Preparation of plant extract:

The aerial parts of *Passiflora foetida* was collected from in and around the Shimoga city of Karnataka and the plant was authenticated by Dr.Krishnaswamy K., Dean and Botanist, Dept. of Biological Science, Sahyadri Science college, Shimoga. The

aerial part of *Passiflora foetida* were shade dried, and reduced to a coarse powder in a pulveriser (Sunbeam, Munger, India) using mesh no.3 and passed through a sieve no.40 to obtain about 1.5 kg of powder. Various extracts of the plant material were prepared by Soxhlet extraction method. The powdered material of *Passiflora foetida* was extracted with the different solvents (Ethanol, Petroleum ether and Ethyl acetate) in a Soxhlet extractor for 48 hrs. in three batches of 35 g each. The extracts were concentrated in vacuum using rotary flash evaporator (Buchi, Flawil, Switzerland). The solvent was removed completely over the water bath and finally dessicator dried. The extracts so obtained were labelled, weighed and the yield calculated in terms of grams percent of the weight of the powdered aerial parts of the plant and is stored in air tight containers. The extracts are then used for the Anti-hypertensive activity.

Phytochemical analysis:

The extracts so obtained from each of the solvents were subjected to the qualitative-chemical tests to detect the major active constituents. The extracts were tested for the presence of Carbohydrates, proteins, tannins

saponins, triterpenoids, flavonoids, phytosterols, quinines, glycosides, alkaloids, fixed oils and fats (Khandelwal 1995 and Kokate C K 2005).

Animals:

Healthy, young adult male and non-pregnant female Swiss albino mice (20-30 g) and rats (150-200 g) of Wistar strain of either sex were used for the acute toxicity and Anti-hypertensive activity. The animals were procured from the Central animal house, National College of Pharmacy, Shimoga, Karnataka. After randomization into Control, standard, and various test groups, animals were acclimitized for a period of ten days under standard husbandry conditions Viz. Room temperature 22° C (\pm 3), Relative humidity (50-60%), Lighting sequence being 12 hrs. light and 12 hrs. dark (OECD Guidelines 425, 2001). All the animals were fed with rodent pellet diet (Gold Mohr, Lipton India Ltd.) and drinking water was allowed *ad-libitum* under strict hygienic conditions. Ethical clearance (NCP/IAEC/CL25/05/2011-12) for performing experiments was obtained from Institutional Animal Ethical Committee (IAEC).

Acute toxicity study:

The method of “up and down” (OECD Guideline No. 425) of CPCSEA was adopted for toxicity studies. The experiments were initiated only after the approval of the Institutional Animal Ethical Committee. Female Albino mice weighing 20-25 g and of 8-12 weeks of age were used to determine the dose. The animals were randomized into control, standard and various test groups, each group containing 6 animals each and were fasted overnight prior to the acute toxicity experimental procedure (OECD Guidelines 425, 2001). Tween-80

(1% v/v) was used as a suspending agent and the extracts were administered orally to each animal in test group as a single dose by gavage using a suitable intubation cannula.

Anti-hypertensive activity:

Healthy adult albino rats of wister strain were divided into five groups each containing six animals each. Systolic and diastolic blood pressure was measured. All the groups were given 8% salt (NaCl) solution for two weeks to induce hypertension (O. Priyanka et al. 2012). After two weeks, the salt- treated animals were assigned the following treatment:

Group 1 (n=6) : Control group received distilled water.

Group 2 (n=6) : Standard group received Amlodipine at a dose (5 mg/kg body weight).

Group 3 (n=6) : Treated group I received ethanol extract at a dose (300 mg/kg body weight).

Group 4 (n=6): Treated group II received Petroleum ether extract at a dose (300 mg/kg body weight).

Group 5 (n=6): Treated group III received Ethyl acetate extract at a dose (300 mg/kg body weight).

Measurement of Blood pressure:

Systolic and diastolic blood pressure was measured before the induction of hypertension, 1st week and 2nd week. After completion of two weeks (14 days) of hypertension induction, on 15th day,

animals were administered respective drugs and the blood pressure was measured in awaked animals after 1 hour and 2 hours by the non-invasive blood pressure module using NIBP pressure meter, LE 5001,

V02/0402L, Panlab, Harvard apparatus, Barcelona, Spain (O. Priyanka et al. 2012).

RESULTS

Phytochemical analysis:

The phytochemical analysis reveals that flavonoids, glycosides, alkaloids,

phytosterols, triterpenoids, carbohydrates, fixed oils and fats are present in the ethanol extract. In ethyl acetate extract flavonoids, alkaloids, glycosides, and carbohydrates are present and in petroleum ether extract phytosterols, triterpenoids, fixed oils and fats are present.

Table No. 1: Table showing the Qualitative Chemical investigations of Extracts of *P. foetida*

Sl. no.	Phytoconstituents	Ethanol extract	Petroleum ether extract	Ethyl acetate extract
1.	Carbohydrates	+	-	+
2.	Proteins	-	-	-
3.	Tannins	-	-	-
4.	Saponins	-	-	-
5.	Triterpenoids	+	+	-
6.	Flavonoids	+	-	+
7.	Phytosterols	+	+	-
8.	Quinones	-	-	-
9.	Glycosides	+	-	+
10.	Alkaloids	+	-	+
11.	Fixed oils and fats	+	+	-

+: Present; -: absent

Acute toxicity study:

Acute toxicity test was carried out in mice. Initially four doses of the each extract that is 500, 1000, 1500, 2000 mg/kg body weight was taken. Dose progression factor was the antilog of 1/(the estimated slope of the dose-

response curve). All groups of test drugs showed neither any toxic effect nor any lethal effect in the dose range of 500 to 2000mg/kg body weight. Hence the dose is increased to 3000mg/kg body weight in which altered behaviour of some mice had

been observed and also caused 50% mortality that is 3 animals died in each group, so this dose was considered as lethal dose. The ethanol, petroleum ether, and ethyl acetate, extracts caused toxicity and / or mortality when administered at a dose of 3000 mg/kg body weight orally. To study the antihypertension activity the extracts were administered in the dose of 300mg/kg body weight which is equal to 1/10th of 3000mg/kg body weight.

Effect on Systolic blood pressure (SBP) and Diastolic blood pressure (DBP):

The changes in SBP and DBP in all the groups before hypertension induction, after 1st and 2nd week of induction and at the end of 2 weeks 1hr after drug administration and 2 hrs after drug administration are given in Table No. 2 and 3. Two weeks of salt in water (8% NaCl) resulted in significant

elevation in SBP and DBP compared to control animals. Administration of *Passiflora foetida* extracts (Ethanol, petroleum ether and ethyl acetate) demonstrated a significant fall in SBP and DBP to varying degrees when compared to control animals.

Effect on Systolic blood pressure (SBP)

The increased levels of systolic blood pressure were significantly lowered in animals treated with ethyl acetate extract when compared to other extracts (Ethanol, and petroleum ether) at the dose of 300 mg/kg body weight. The ethyl acetate extract has been shown a significant protective effect, whereas ethanol and petroleum ether extracts were shown moderate and less significant protective effects when compared to control and standard.

Table No. 2: Effect on Systolic blood pressure before hypertension induction, during 2 weeks of induction and at the end of the 2nd week after drug administration

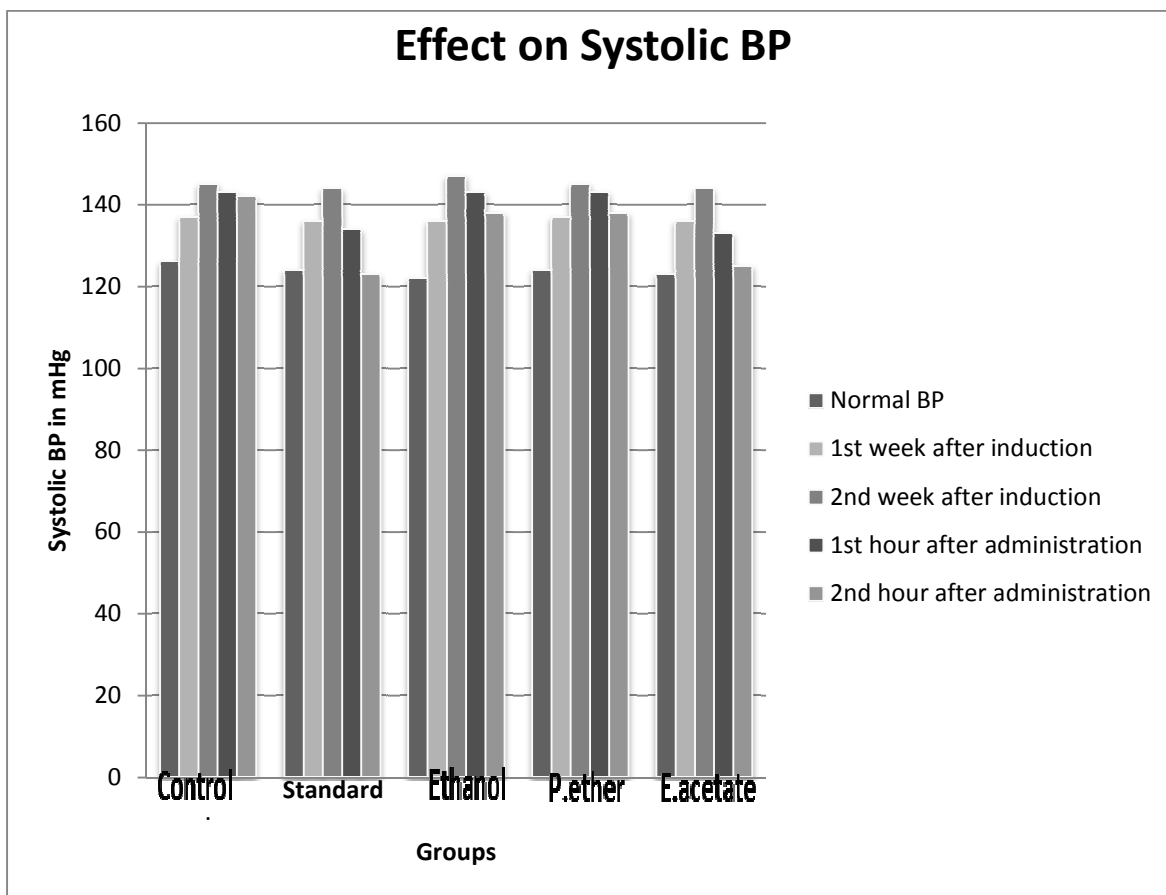
Systolic Blood Pressure (in mmHg) during induction of hypertension (2 weeks)			Blood Pressure (in mmHg) after drug administration (After 2 weeks)		
Before	1 st week	2 nd week	Treatment	1 st hour	2 nd hour
126±0.60	137±0.45***	145±1.33***	Water by <i>p.o</i> route	143±0.91	142±0.75
124±1.15	136±0.91***	144±1.14***	Amlodipine (5 mg/kg) by <i>p.o.</i> route	134±0.80***	123±0.75***
122±1.50	136±0.95***	147±1.13***	Ethanol extract (300 mg/kg b.w) by <i>p.o.</i> route	143±1.42*	138±1.55*
124±1.63	137±0.45***	145±1.23***	Pet.ether extract (300 mg/kg b.w) by <i>p.o.</i> route	143±1.42**	138±1.55*
123±1.36	136±0.61***	144±1.23***	Ethyl acetate extract (300 mg/kg) by <i>p.o.</i> route	133±0.84***	125±1.53***

Note: Data was analysed using one way ANOVA followed by pairwise comparison.

Values are expressed as mean ± S.E.M. *n* = 6,

****P*<0.001, ***P*< 0.01 and **P*< 0.05.

Figure No. 1: Histogram showing the effect of *Passiflora foetida* extracts on systolic BP



Effect on Diastolic Blood Pressure (DBP):

The increased levels of diastolic blood pressure were significantly lowered in animals treated with ethyl acetate extract when compared to other extracts (ethanol, and petroleum ether) at the dose of 300 mg/kg body weight. The ethyl acetate

extract has been shown a significant protective effect, whereas ethanol and petroleum ether extracts showed less significant protective effects when compared to control.

Table No. 3: Effect on Diastolic blood pressure before hypertension induction, during 2 weeks of induction and at the end of the 2nd week after drug administration

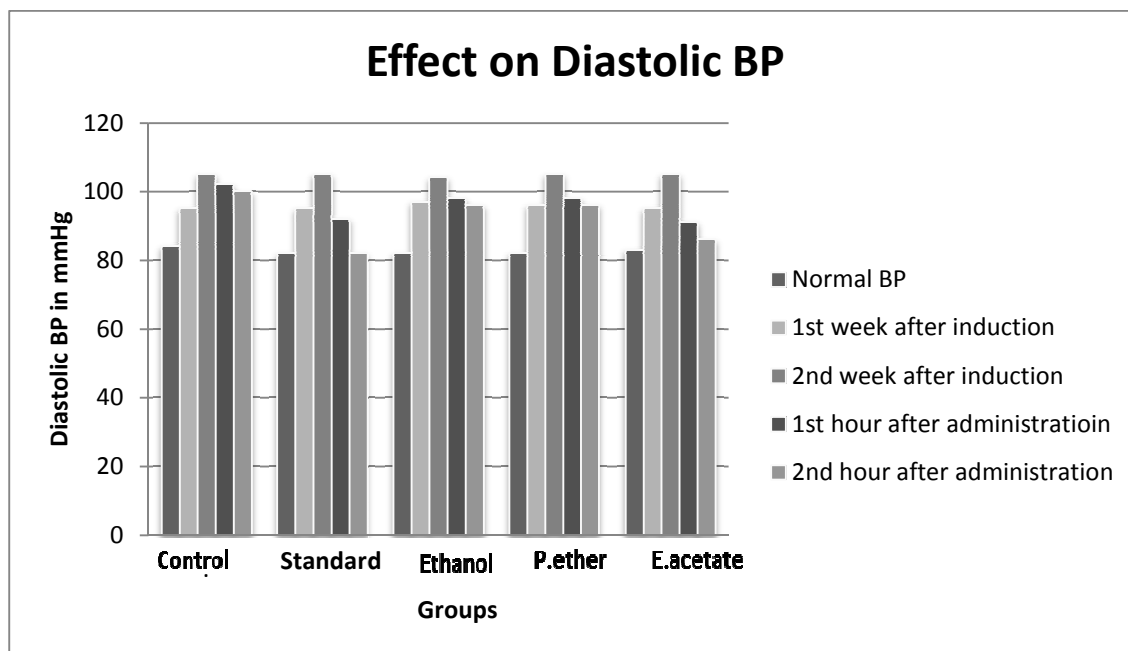
Diastolic Blood pressure (in mmHg) during induction of hypertension (2 weeks)			Blood Pressure (in mmHg) after drug administration (after 2 weeks)		
Before	1 st week	2 nd week	Treatment	1 st hour	2 nd hour
84±0.60	95±0.99***	105±1.21***	Water by <i>p.o.</i> route	102±1.12	100±1.47
82±0.45	95±0.86***	105±0.98***	Amlodipine (5 mg/kg) by <i>p.o.</i> route	92±1.86***	82±0.61***
82±0.86	97±0.99***	104±1.68***	Ethanol extract (300mg/kg b.w) by <i>p.o.</i> route	98±0.89*	96±0.75*
82±1.09	96±0.95***	105±1.59***	Pet.ether extract (300 mg/kg b.w) by <i>p.o.</i> route	98±0.89*	96±0.92*
83±1.12	95±1.13***	105±1.13***	E. acetate extract (300 mg/kg) by <i>p.o.</i> route	91±0.40***	86±1.73***

Note: Data was analysed using one way ANOVA followed by pairwise comparison.

Values are expressed as mean ± S.E.M. *n* = 6,

***P<0.001, **P< 0.01 and *P< 0.05.

Figure No. 2: Histogram showing the effect of *P. foetida* extracts on Diastolic BP



DISCUSSION

Flavonoids have been shown vasodilator effects in isolated aortas stimulated with noradrenaline, KCl or phorbol esters and these effects are independent of the presence of endothelium which contains EDRF (Endothelium Relaxation Factor). Thus, the direct vasodilator effect might be contribute to the antihypertensive effects of ethyl acetate extract used in the present study as the phytochemical investigation of the extract, revealed the presence of Flavonoids, glycosides, alkaloids, phytosterols, triterpenoids, carbohydrates, fixed oils and

fats. Previous studies suggest that Flavonoids act by decreasing the oxidative stress (Suzuki H et al. 1995). Urinary levels of isoprostane F_{2a} , a prostaglandin-like compound produced in a non-enzymatic reaction of arachidonic acid and superoxide, as well as plasma levels of total plasma malondialdehyde have been proposed to be reliable markers of lipid peroxidation and oxidative stress (Marrow J.D et al. 1966). Increased superoxide anion production is thought to contribute to hypertension. In fact, reduction of superoxide anions with alloxanthine, an inhibitor of xanthine

oxidase, or CuZn superoxide dismutase acutely decreases the mean arterial pressure (Kitts D.D et al. 1998). One possible mechanism for the hypertensive effects of superoxide may be that this free radical can inactivate NO and, therefore, impair the NO-dependent vasodilation. In addition, some products of oxidative stress, such as 8-iso-prostaglandin F_{2α}, have been shown to be potent vasoconstrictors and raise blood pressure. Furthermore, excess active oxygen species may also stimulate cellular proliferation by direct mitogenic effects on vascular smooth muscle cells and by decreasing the effective concentrations of

NO and Prostacyclin (Grunfeld S et al. 1995).

CONCLUSION

The Anti-hypertensive effect produced by ethyl acetate extract of *Passiflora foetida* may be due the activation of NO-dependent vasodilation and due to the presence of flavonoids in the ethyl acetate extract. Hence it can be concluded that the ethyl acetate extract of *Passiflora foetida* has the potential to become a good Anti-hypertensive drug; further studies are needed to know the exact mechanism of action.

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Conflict of Interest statement

The authors report no conflict of interest.