

Full Length Research Paper

Antihypertensive action of ethanolic extract of *Imperata cylindrica* leaves in animal models

E. E. Mak-Mensah^{1*}, G. Komlaga² and E. O. Terlabi¹

¹Department of Biochemistry and Biotechnology, Kwame Nkrumah University of Science and Technology (KNUST), Kumasi, Ghana.

²Department of Pharmacognosy, KNUST, Kumasi, Ghana.

Accepted 10 June, 2010

This work was carried out to determine antihypertensive effect of ethanolic extract of *Imperata cylindrica* leaves using cat and rabbit models. A freeze dried 70% ethanolic extract was prepared and increasing concentrations from 10 to 320 mg/ml were administered *in vivo* to anaesthetized cats and *in vitro* to rabbit jejunum. Adrenaline and phentolamine were used as control drugs. The extract exhibited a significant dose-dependent reduction in amplitude of contraction of smooth muscle cells of rabbit jejunum in comparison with standard antihypertensive drug, adrenaline. The heart pressure of the cats was significantly reduced with 160 and 320 mg/ml of the extract from 266 to 180 mmHg ($p = 0.012$) but there was no effect on the heart rate. The minimum effective dose EC_{50} was 0.013. Ethanolic leaf extract of *I. cylindrica* exhibited vasodilative antihypertensive properties similar to mechanism of action of adrenaline. The extract could be used to manage hypertension.

Key words: *Imperata cylindrica*, antihypertensive, ethanolic extract, β -blockers.

INTRODUCTION

Hypertension is the most common disease in industrialized nations, with prevalence above 20% in the general population (Adrogué and Madias, 2007). Its prevalence is predicted to increase by 60% by 2025, when a total of 1.56 billion people may be affected (Kearney et al., 2005). In Ghana, the prevalence of hypertension is 29 - 30% (Cappuccio et al., 2004) which shows that comparatively, the disease prevalence in the developing countries is the same as in the developed ones (Whitworth, 2003).

One of the classes of antihypertensive drugs is β -adrenergic receptor blocker (β -blockers). β -blockers are competitive inhibitors of adrenaline and norepinephrine for the β -adrenoceptors thereby inhibiting their sympathetic effect. β -adrenoceptors are located in cardiac nodal tissues, conducting systems and contracting myocytes. Rabbit jejunum has α -adrenoceptors while the heart has both β_1 and β_2 adrenoceptors. The mode of

action of β -blockers is the inactivation of adenylyl cyclase which produces cAMP from ATP. The fall in cAMP levels also inactivates cAMP dependent protein kinase which would have otherwise phosphorylated L-type calcium channels. In effect the influx of calcium into the sarcoplasm is curtailed reducing contractility (http://en.wikipedia.org/wiki/beta_blocker).

Phentolamine is a non-selective α adrenoceptor antagonist drug (Rang et al., 2003) that binds to the receptors causing inhibition of any agonist drug of the same receptors. It binds to and stimulates α - adrenoceptor to initiate release of calcium ions causing vasoconstriction. *Imperata cylindrica* is the scientific name for spear grass. It belongs to the Poales order of the Kingdom Plantae, family; poaceae.

The spear grass is a perennial, rhizomatous grass that grows from 2 to over 4 feet in height. (http://en.wikipedia.org/wiki/Imperata_cylindrica).

Alternate medicine in the form of plant medicine is gaining popularity due to its low and mild side effects and its affordability (WHO, 2003). The rhizome of the *I. cylindrica* was found to contain two lignans namely

*Corresponding author. E-mail: eemakmensah@gmail.com.

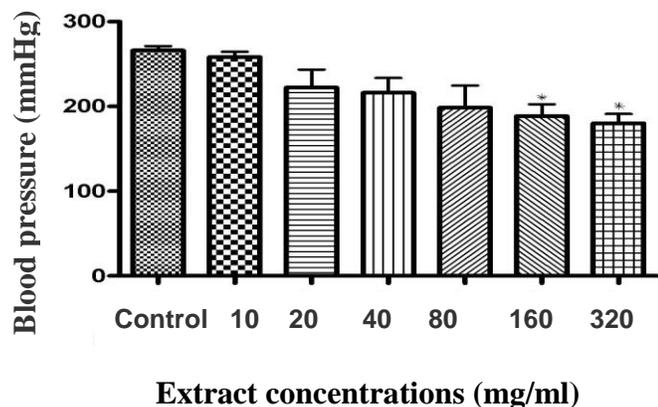


Figure 1. Blood pressure against increasing concentrations of Extract on anaesthetized cat. Control = No extract - initial blood pressure before administration of extract. concentrations showing significant difference, $p < 0.05$.

Table 1. Dose concentration and blood pressure responses of the cat to the extract.

Concentration (mg/ml)	Blood pressure (mmHg)
Control	266.00 ± 5.29
10	258.00 ± 6.17
20	222.33 ± 21.40
40	216.00 ± 17.50
80	198.67 ± 25.98
160	188.67 ± 13.92
320	180.00 ± 11.02

Mean ± SEM, control = No extract was added.

Graminones A and B. The IR spectra of these molecules displayed absorption bands at 1775 cm^{-1} , indicating the presence of a γ lactone in the molecules. Graminone B has further been identified to have an inhibitory action on rabbit aorta (Matsunaga et al., 1994). However, no work has been done to ascertain similar properties in the leaf which forms the bulk of the grass. The purpose of this study is to determine whether the leaf extract of *I. cylindrica* has an anti-hypertensive property, reduction of blood pressure in cat and vasodilative effect on smooth muscle of rabbit jejunum.

MATERIALS AND METHODS

Animals

All the animals were kept in a room under environmentally controlled temperature of $24 \pm 1^\circ\text{C}$ and a 12h light – 12h dark cycle. They were acclimatized at least once a week before starting the experiments. The animal experimentation was carried out in an ethically proper way by following guidelines as set by the world health organization.

Plant material

Leaves of *imperata cylindrica* were collected from the faculty of horticulture agriculture on knust campus and authenticated by a curator at the department of theoretical and applied biology herbarium knust.

Preparation of extract

The leaves of the plant were air dried for a week. The dried leaves were ground into powder and mixed with 70% ethyl alcohol. The mixture was then kept for three days at room temperature. On the third day the mixture was stirred and left overnight and afterwards filtered. The alcohol was evaporated by the use of a water bath and the extract kept in a desiccator pending use.

In vitro studies on effect of extract on smooth muscle

Five adult male rabbits were sacrificed and their jejunum cut helically into strips of 3 - 4 cm. Each strip was mounted in a 10 ml organ bath containing Ringer-locke's solution of composition (mM): NaCl, 155.0; KCl, 5.7; NaHCO_3 , 6.0; CaCl_2 , 1.0 and glucose, 5.5 at 37°C and gassed with 95% O_2 and 5% CO_2 . The strips of the rabbit jejunum was placed under an initial passive tension and allowed to equilibrate. Contractile responses of the jejunum strips to doses of 10, 20, 40, 80, 160 and 320 mg/ml of the extract and adrenaline were recorded, with use of 40 $\mu\text{g/ml}$ of the phentolamine kept constant, using a force displacement transducer (model A-6360; Harvard Apparatus Ltd, Kent, England) coupled to an oscillograph (model 50 - 8622, Harvard Apparatus Ltd, Kent, England) as used by Woode et al. (2008).

In vivo studies on effect of extract on anaesthetized cat

Five cats were anaesthetized with a 5 ml/kg mixture of urethane (10%) and chloralose (1%) given intraperitoneally, mounted and prepared for recording of blood pressure and heart rate. Filtered extract were then injected into the femoral vein. Using a polythene tubing, arterial blood pressure was recorded through a pressure transducer and the phasic arterial pressure monitored by a universal oscillograph.

RESULTS

Figure 1 shows the effect of the extract on the mean arterial blood pressure of the anaesthetized cats which decreases from the control with a high value of 266 to 180 mmHg on administration of 320 mg/ml of the extract. The data in the Tables 1 and 2 were used to draw their corresponding figures. The graph for the effect of the extract on the mean heart rate of the anaesthetized cat (Figure 2) remained constant at a value of 39 beats/min upon administration of 0 to 320 mg/ml of the extract. Figure 3 shows a steep slope for the effect of adrenaline on rabbit jejunum. The effect of adrenaline rose from 0 to almost 100% maximum response. The graph for the extract rose steadily from 0 to almost 50% of maximum response. The presence of phentolamine did not affect the response of the rabbit jejunum to the extract appreciably (Table 3) $p > 0.05$. The extract may be phentolamine agonist. Adrenaline and phentolamine are

Table 2. Dose concentrations and heart rate responses of the cat to the extract.

Concentration (mg/ml)	Heart Rate (Beats/min)
Control	39.00 ± 3.06
10	39.00 ± 3.06
20	39.00 ± 3.06
40	39.00 ± 3.06
80	39.00 ± 3.06
160	39.00 ± 3.06
320	39.00 ± 3.06

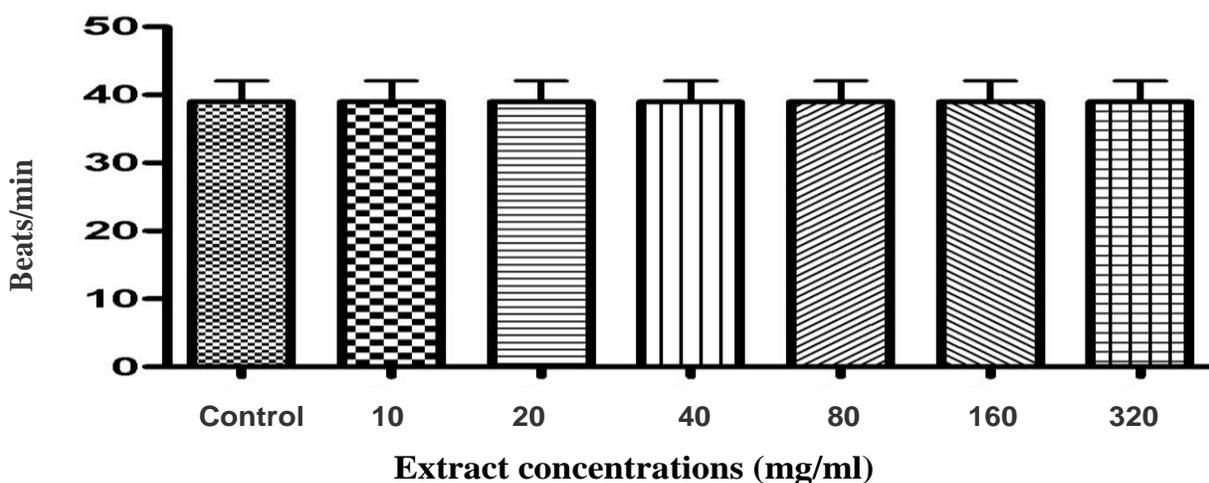


Figure 2. Heart rate against increasing concentrations of extract on anaesthetized cat.

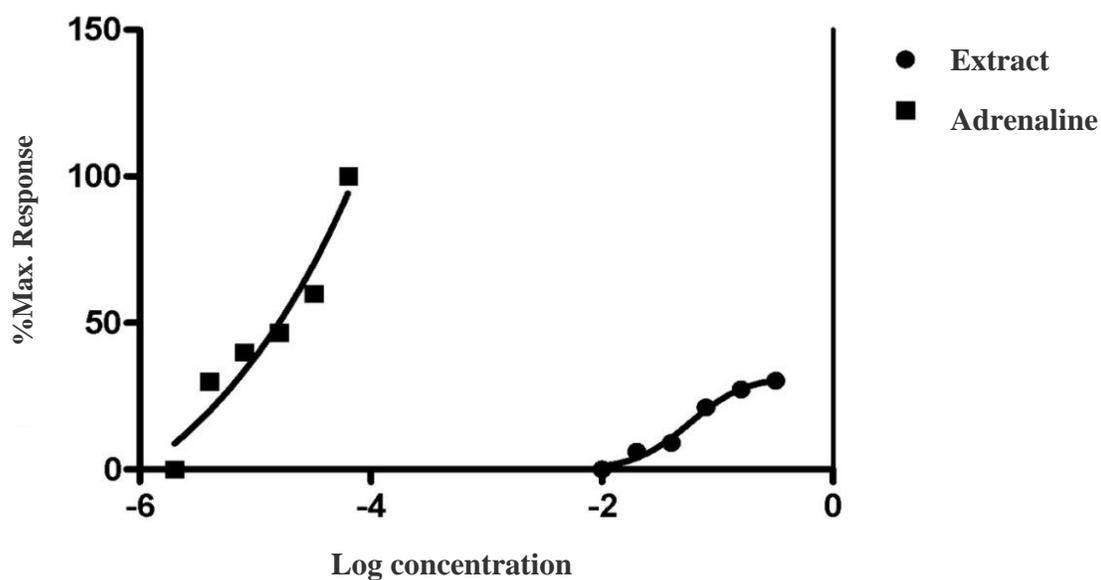


Figure 3. A comparison of percentage maximum response against log dose concentration of adrenaline and extract on rabbit jejunum.

Table 3. Dose concentrations and mean responses of the rabbit jejunum to the extract and phentolamine.

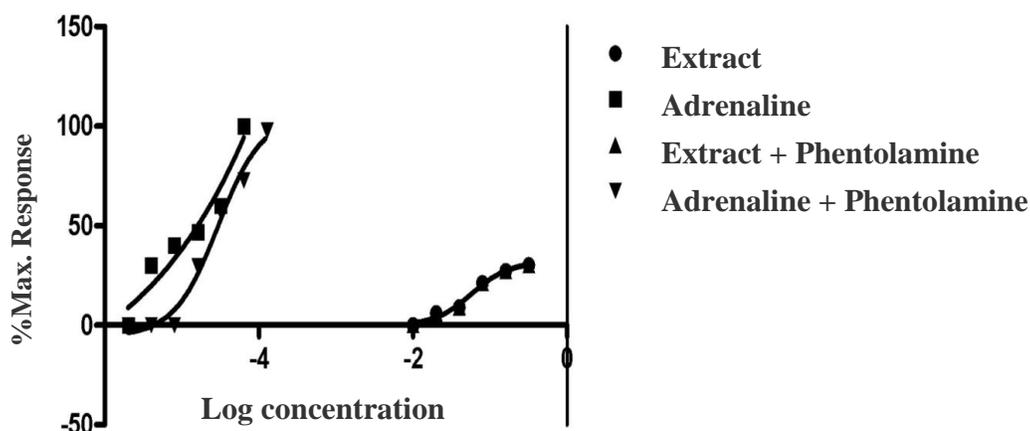
Concentration of extract (mg/ml)	A/(mm)	B/ (mm)
0.010	10.50 ± 0.29	11.00 ± 0.29
0.020	13.50 ± 0.29	13.00 ± 0.29
0.040	15.00 ± 0.29	14.00 ± 1.04
0.080	17.50 ± 0.30	18.00 ± 0.58
0.160	20.50 ± 0.28	20.00 ± 0.50
0.320	23.00 ± 1.04	21.00 ± 0.58

A= Contraction of jejunum in the presence of the various concentration of the extract. B= Contraction of jejunum in the presence of varying concentrations of the extract with constant concentration of phentolamine (40 µg/ml). The extract worked like phentolamine (β-blocker).

Table 4. Dose concentrations and mean responses of the rabbit jejunum to adrenaline and phentolamine.

Concentration of adrenaline (µg/L)	C (mm)	D (mm)
2	19.00 ± 0.29	0.00 ± 0.00
4	24.50 ± 0.50	0.00 ± 0.00
8	29.00 ± 0.50	0.00 ± 0.00
16	32.50 ± 0.50	13.00 ± 0.29
32	36.50 ± 0.00	25.00 ± 0.50
64	43.00 ± 0.50	32.00 ± 0.58

Contraction of jejunum (mm) in the presence of varying concentrations adrenaline=C, D= Contraction of jejunum in the presence of various concentrations of adrenaline with constant concentration of phentolamine.

**Figure 4.** Mean response of rabbit jejunum to antagonism between extract, adrenaline and phentolamine.

antagonists as seen in Table 4.

Figure 4 shows antagonism between adrenaline and phentolamine causing a rightward shift from the original graph of adrenaline with the percentage maximum response remaining the same at almost 100%. The graph

of the extract and phentolamine remained the same as that of the extract alone with a percentage maximum response below 50%. Figures 5 and 6 show the vasodilative properties of both extract and control drug, adrenaline. The contraction of jejunum was more

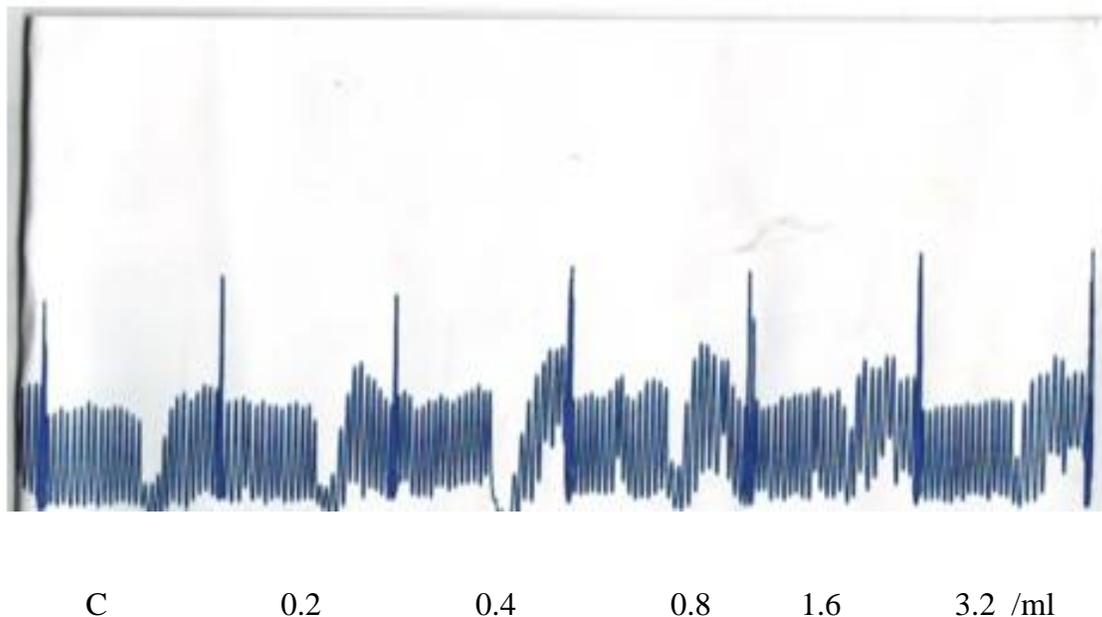


Figure 5. Contraction of rabbit jejunum in response to increasing doses of the extract. C= control (volume of extract was 0.00 ml). Increasing volume of extract from 0.2 to 3.2 ml

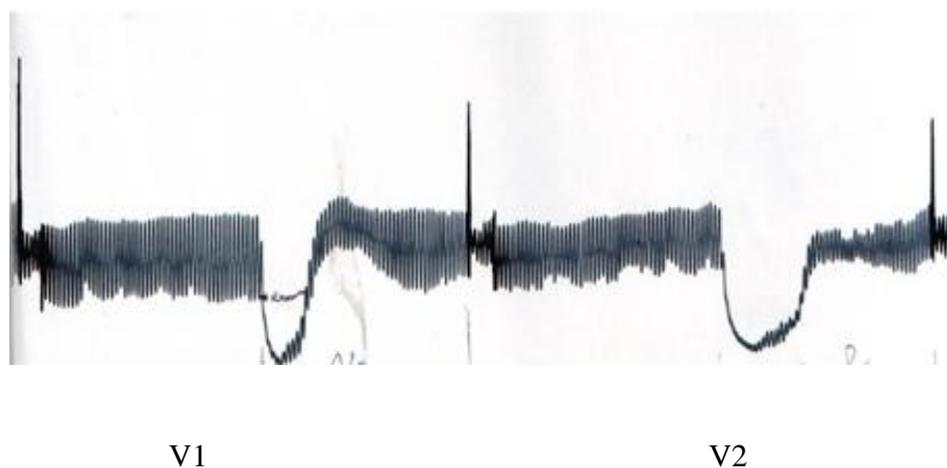


Figure 6. Effect of adrenaline on rabbit jejunum, volume of adrenaline administered V1 and V2 are respectively 0.4 and 0.8 ml.

pronounced in the presence of adrenaline than the crude extract at the same volume, 0.4 and 0.8 ml. This is shown by the differences in the troughs in Figures 5 and 6.

DISCUSSION

The graph representing the mean blood pressure against increasing concentration of the *I. cylindrica* leaf extract on

anaesthetized cat (Figure 1) showed a significant decrease from a mean control reading with p-value of 0.012 from 266 to 180 mmHg upon the administration of 320 mg/ml of the extract. This may be due to active antihypertensive component of the extract acting on adrenoreceptors present on the vascular vessels of the anaesthetized cat. It could also be inferred that at extract concentration of 320 mg/ml, a greater number of the adrenoreceptors had been acted upon by the active

component to illicit the drop in blood pressure. There was no significant reduction however, in the mean arterial blood pressure till administration of 160 mg/ml of the extract. This observation is attributed to the fact that the extract concentrations of 10, 20, 40 and 80 mg/ml do not contain sufficient amount of the active component to cause a significant reduction in the arterial blood pressure. Beyond 320 mg/ml the extract was unable to cause any significant reduction in blood pressure (data not shown). This may be due to saturation of receptor sites for the extract. The effect of increasing concentrations of the extract on the heart rate of the anaesthetized cat showed no significant variation (Figure 2), an indication of inability of the extract to act on the receptors of the heart. It may be that the extract acted on the receptors found in only the vascular tissues.

The comparison of percentage maximum response against log dose concentration of adrenaline and the extract showed difference in level of action on the rabbit jejunum (Figure 3). There was a steep rise for adrenaline while the extract was very steady but short (Figure 3). The steady rise began from log concentration of negative 2 and marginally fell short of the 50% maximum response. This result could be due to inhibition of the sodium pump intracellularly, probably due to a calcium blocker binding to protein of the calcium channel (Iwamoto, 2006) inhibiting calcium movement. Again the similarity in the nature of both curves may be an indication of both adrenaline and the extract acting on the same kind of receptors (Figures 5 and 6).

The antagonistic test using phentolamine and the extract on the rabbit jejunum showed no significant response of the extract to an antagonistic drug (Figure 4). This is because the antagonistic drug phentolamine was able to stimulate alpha adrenoceptors which leads to the release of Ca^{2+} thus leading to vasoconstriction which nullifies the effect of the vasodilative extract even on administration of increasing concentrations. However, with adrenaline, the effect of the phentolamine is overcome with increasing concentrations showing a shift from the dose response curve of the adrenaline alone on the smooth muscle tissue (Figure 4). This may be due to the potency of adrenaline as compared to the extract.

Vasodilation was not observed upon administering adrenaline and phentolamine to the tissue until 16 μ g/ml (Table 4). This is due to competitive antagonism between adrenaline and phentolamine resulting in the displacement of phentolamine molecules from the receptor sites. This effect was not seen with the extract alone and the extract plus phentolamine (Table 3) as they resulted in curves superimposed on each other (Figure 4). This could be due to blocking of the receptor sites by the extract preventing phentolamine from binding. The higher the EC_{50} values of a drug, the less potent the drug and vice versa. From the graph (Figure 4) it is indicated that adrenaline is more potent than the extract. Both the extract and adrenaline produced similar vasodilative

effects on rabbit jejunum, the latter produced more pronounced effect (Figures 5 and 6) indicated by bigger troughs (Figure 6). This could be attributed to the more refined nature of the adrenaline than the crude form of the extract which might be hindering its efficacy.

Conclusion

I cylindrica leaf extract exhibited significant antihypertensive action by relaxing and dilating smooth muscles of blood vessels (*in vivo*) and gastrointestinal tract (*in vitro*). But there was no significant change in the cardiac output. The effective dose of the extract was found between the range of 0.024 and 0.136 mg/ml. The extract when refined can be used in the management of hypertension.

ACKNOWLEDGEMENTS

The authors would like to thank Messrs S. N. Darko and L. Kusi of the Department of Biochemistry and Biotechnology (KNUST) as well as T. Ansa and E. Boakye of Department of Pharmacology (KNUST) for the roles they played in the experimentation of this work.

REFERENCES

- Adroque HJ, Madias NE (2007). Sodium and Potassium in the Pathogenesis of Hypertension. *N. Eng. J. Med.*, 356: 1966-1978.
- Cappuccio FP, Micah FB, Emmett L, Kerry SM, Antwi S, Martin-Peprah R, Phillips RO, Phange-Rhule J, Eastwood JB (2004). Prevalence, detection, management, and control of hypertension in Ashanti, West Africa. *Hypertension*, 43: 1017-1022.
- http://www.who.int/mediacentre/factsheets/fs_134/en/.
- http://en.wikipedia.org/wiki/beta_blocker.
- http://en.wikipedia.org/wiki/Imperata_cylindrica.
- Iwamoto T (2006). Vascular Na^+/Ca^{2+} exchanger: Implications for the pathogenesis and therapy of salt-dependent hypertension. *Am. J. Physiol. Regul. Integr. Comp. Physiol.*, 290: 536- 545.
- Kearney PM, Whelton M, Reynolds K, Muntner P, Whelton PK, He J (2005). Global burden of hypertension: analysis of worldwide data. *Lancet*, 365: 217-223.
- Matsunaga H, Shibuya M, Ohizuma Y (1994). Graminone B, a novel lignan with vasodilative activity from *Imperata cylindrica*. *J. Nat. Prod.*, 57: 1734-1736.
- Rang HP, Dale MN, Ritter JM, Moore PK (2003). *Pharmacology*. 5th edition, Churchill Livingstone, London, pp 140, 173, 179, 298-299.
- Whitworth JA (2003). 2003 World Health Organization (WHO)/International Society of Hypertension (ISH) statement on management of hypertension. *J. Hypertens.*, 21: 1983-1992.
- WHO (2003). Traditional Medicine URL: Fact Sheet p. 134.
- Woode E, Amisah F, Duwiejua M, Ansa C, Fleischer TC, Sawyer IK (2008). Effects of *Tetrapleura Tetraptera* (Taub) fruit extract on some isolated tissues: possible mechanisms of antihypertensive action. *J. Sci. Technol.*, 28: 23-34.