REVIEW OF LITERATURE
HYPERTENSION

All our knowledge of cardiovascular disease can be traced back to Harvey's fundamental discovery of the circulation of the blood. This was an early product of the scientific revolution as a result of which appeal to authority was replaced by original observation and experiment that is epitomised in Harvey's exhortation to "search and study out the secret of nature by way of experiment" (Salvetti, 1996).

The first major fruit of the experimental approach to blood pressure research was the result of work carried out by a scientific genius who was not a doctor but a clergyman Reverend Stephan Hales, by inserting a brass tube in the artery of a horse he was able to estimate blood pressure by the height of the pulsating column of blood (Swales, 1999).

Definition and classification of Hypertension

The continuous relationship between the level of blood pressure and the risk of cardiovascular events, and the arbitrary nature of the definition of hypertension have contributed to the variation in the definitions issued by various national and international authorities. WHO ISH guidelines emphasize that the decision to lower the elevated pressure in a particular patient is not based on the level of blood pressure alone but on assessment of the total cardiovascular risk in that individual.
Definition

Hypertension is defined as a systolic blood pressure of 140 mm Hg or greater and/or a diastolic blood pressure of 90 mm Hg or greater in subjects who are not taking antihypertensive medication.

Definitions and classification of blood pressure levels

When a patient's systolic and diastolic blood pressures fall into two different categories the higher category should apply.

<table>
<thead>
<tr>
<th>Category</th>
<th>Systolic (mmHg)</th>
<th>Diastolic (mmHg)</th>
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<tbody>
<tr>
<td>Optimal</td>
<td>&lt; 120</td>
<td>&lt; 80</td>
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<tr>
<td>Normal</td>
<td>&lt; 130</td>
<td>&lt; 85</td>
</tr>
<tr>
<td>High-Normal</td>
<td>130-139</td>
<td>85-89</td>
</tr>
<tr>
<td>Grade 1 Hypertension (&quot;mild&quot;)</td>
<td>140-159</td>
<td>90-99</td>
</tr>
<tr>
<td>Subgroup: Borderline</td>
<td>140-149</td>
<td>90-94</td>
</tr>
<tr>
<td>Grade 2 Hypertension (&quot;moderate&quot;)</td>
<td>160-179</td>
<td>100-109</td>
</tr>
<tr>
<td>Grade 3 Hypertension (&quot;severe&quot;)</td>
<td>≥ 180</td>
<td>≥ 110</td>
</tr>
<tr>
<td>Isolated Systolic Hypertension</td>
<td>≥ 140</td>
<td>&lt; 90</td>
</tr>
<tr>
<td>Subgroup: Borderline</td>
<td>140-149</td>
<td>&lt; 90</td>
</tr>
</tbody>
</table>

Who-ISH guidelines (1999) for management of hypertension
Etiopathogenesis of Hypertension

The cause of elevated arterial pressure is unknown in over 90% cases. Patients with arterial hypertension and no definable cause are said to have primary, essential or idiopathic hypertension while those individuals with a specific organ defect responsible for hypertension are said to have secondary form of hypertension.

Genetic factors have long been assumed to be important in the genesis of hypertension. Extensive studies have concluded that the level of B.P is determined by a large number of genes, with a multiplicity of mechanism.

In the mean time current estimates suggest that in western societies genetic factors account for about 30% of the population variability in B.P. A number of environmental factors have been implicated in the development of hypertension including salt intake, obesity, occupation, family size and over crowding. Of these salt intake has received the greatest attention. It is estimated that approximately 60 per cent of hypertensives are particularly responsive to the level of sodium intake.

Age, race, sex, smoking, serum cholesterol, glucose intolerance and obesity may all alter the prognosis of this disease. The younger the patient when hypertension is first noted, the greater the reduction in life expectancy if left untreated. Urban blacks have twice the prevalence rate for hypertension as whites. Females with hypertension run the same relative risk of a morbid cardiovascular event compared to their normotensive counterparts as males do (Williams, 1991) Independent risk factors like an elevated serum cholesterol, glucose intolerance and/or
cigarette smoking, significantly enhance the effect of hypertension on mortality rates regardless of age, sex or race.

There is a positive correlation between obesity and hypertension. A gain in weight is associated with an increased frequency of hypertension in subjects with normal pressure.

Natural History: Blood pressure is the product of cardiac output and total peripheral resistance (TPR). Experimental studies in animals and man have suggested that during the development of hypertension, there had been an early phase of high cardiac output and normal total peripheral resistance which changes over several weeks/months to a pattern of normal cardiac output and high TPR. This lead to the formulation of autoregulatory theory of hypertension (Korner, 1994) According to this theory, early retention of sodium and water leads successively to an increase in blood volume, in cardiac filling pressures and to an increase in cardiac output which is initially responsible for raised blood pressures.

The high cardiac output perfuses the peripheral tissues in excess of their metabolic requirements resulting in a normal autoregulatory response which are responsible for increasing contribution through raised TPR.

The time over which this transformation develops is similar to the course of development of the structural changes that were first described as an adaptation to hypertension. These changes include medial hypertrophy of the smooth muscle of the larger resistance vessels and luminal narrowing so that wall thickness to lumen ratio is increased.
A subset of patients develop predominant arteriolopathy, characterised by endothelial injury, intimal thickening and ultimately arteriolar occlusion. This is the pathological basis of syndrome of malignant hypertension.

Effects of Hypertension

Patients with hypertension die prematurely; the most common cause of death is coronary artery disease, stroke, congestive heart failure and renal failure.

Effects on heart

Cardiac compensation for the excessive work load imposed by increased systemic pressure is at first sustained by concentric left ventricular hypertrophy, ultimately the chamber dilates and signs and symptoms of heart failure appear.

Neurologic Effects

Retinal changes in hypertension are characterised by appearance of hemorrhages exudates, papilloedema, scotomata and even blindness.

Central nervous system dysfunction also occurs frequently in patients with hypertension. Occipital headache (mostly in mornings), dizziness, tinnitus and vertigo are very common.

Cerebral infarction secondary to atherosclerosis is commonly observed (Williams, 1998). Cerebral haemorrhage is the result of both the elevated arterial pressure and the development of cerebral vascular microaneurysms.
Renal effects: Arterio sclerotic lesions of the afferent and efferent arterioles and glomerular capillaries are most common renal vascular lesions in hypertension which can ultimately result in renal failure (Williams, 1998).

Apart from these effects, the incidence of peripheral vascular disease increases markedly in patients with high blood pressure.

TREATMENT OF HYPERTENSION

Goals of Treatment

The primary goal of treatment of the patient with high blood pressure is to achieve the maximum reduction in the total risk of cardiovascular morbidity and mortality (Zanchetti et al., 1993).

This requires

a. Treatment of all the reversible risk factors identified such as smoking, raised serum cholesterol or diabetes.

b. Appropriate management of associated clinical conditions.

c. Treatment of the raised blood pressure per se.

The relationship between cardiovascular risk and blood pressure is continuous. The goal of anti hypertensive therapy should be to restore blood pressure levels defined as normal or optimal. A major determinant of the risk reduction conferred by anti hypertension therapy is the level of blood pressure reduction achieved (Isles et al., 1986).

Comparison of outcomes between the three randomised blood pressure target groups in the HOT study (DBP < 90, 85 or 80 mm hg) confirm that
there is no increase in cardiovascular risk in the patients randomised to the lowest target group (DBP < 80 mm Hg). Among diabetic patients in the HOT study there were significantly lower risk of cardiovascular disease in those patients assigned to the lowest blood pressure target.

Similarly, the results of the UK prospective diabetes study (UK PDS, 1998) demonstrated that tight BP control was associated with a substantial reduction in the risk of major cardiovascular events.

**STRATIFICATION OF RISK**

Stratification of risk to quantify prognosis

Risk strata (typical 10 year risk of stroke or myocardial infarctional: Low risk = less than 15%, medium risk = about 15 - 20% risk, high risk about 20-30%, very high risk - 30% or more.

**TOD** – Target Organ Damage

**ACC** – Associated Clinical Conditions, including clinical cardiovascular disease or renal disease.

**WHO-IISH guidelines (1999) for management of hypertension**

<table>
<thead>
<tr>
<th>Other Risk Factors &amp; Disease History</th>
<th>Blood Pressure (mmHg)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Grade 1 (Mild hypertension) SBP 140-159 or DBP 90-99</td>
</tr>
<tr>
<td>I. No Other risk factors</td>
<td>LOW RISK</td>
</tr>
<tr>
<td>II. 1-2 Risk factors</td>
<td>MED Risk</td>
</tr>
<tr>
<td>III. 3 or more risk factors of TOD or diabetes</td>
<td>High risk</td>
</tr>
<tr>
<td>IV. ACC</td>
<td>V High Risk</td>
</tr>
</tbody>
</table>
It has been found that day time values provided by home or ambulatory blood pressure measurements are on an average around 10-15 mm Hg lower for systolic blood pressure and 5-10 mm Hg lower for diastolic blood pressure. Treatment goals should therefore be modified appropriately.

Management Strategy

Physician, after having assessed the patient stratifies the patient into low, medium high or very high risk of cardiovascular disease events.

<table>
<thead>
<tr>
<th>1. High Very High Risk Group</th>
<th>Institute immediate drug treatment for hypertension and other risk factor or conditions</th>
</tr>
</thead>
<tbody>
<tr>
<td>3. Low risk</td>
<td>Observe the patient over a significant period of time (several months) before deciding whether to institute drug treatment.</td>
</tr>
</tbody>
</table>

Having decided on the broad strategy for management, the physician should then determine the specific therapeutic goals for the individual patient.

1. Monitoring: Of blood pressure and other risk factors

2. Life style measures: To lower the blood pressure and control the other risk factors.
3. Drug treatment: To lower blood pressure and to control the other risk factor and clinical conditions present.

   i. Life style measure – (Non pharmacological Treatment)

1. To lower the blood pressure in the individual patient.

2. To reduce the need for anti hypertensive drugs and maximize their efficacy.

3. To address the other risk factors present.

4. For primary prevention of hypertension and associated cardiovascular Disorders in population.

1. Smoking Cessation

   It is the single most powerful lifestyle measure for the prevention of both cardiovascular and non cardiovascular disease in hypertensive patients. Appropriate counselling and nicotine replacement therapy should be given.

2. Weight reduction

   It is the most important factor predisposing to hypertension. Weight reduction of as little as 5 kg reduces blood pressure in a large proportion of hypertensive individuals.

   The B.P. lowering effects of weight reduction may be enhanced by simultaneous increase in physical exercise. Weight loss of at least 5 kg should be recommended in the first instance, with further increments of 5 kg depending upon the response and the patient's weight.
3. Moderation of Alcohol Consumption

Alcohol intake of up to 3 standard drinks a day may lower the risk of CHD (WHO-ISH guidelines for the management of hypertension, 1999). Otherwise there is a linear relationship between alcohol consumption and blood pressure levels. Accordingly hypertensive patients who drink alcohol should be advised to limit their consumption to no more than 20-30 gm of ethanol per day for men and no more than 10-20 gm of ethanol per day for women. They should be warned against the heightened risk of stroke associated with binge drinking.

4. Reduction in salt intake

Dietary salt intake is a contributor to blood pressure elevation and to the prevalence of hypertension. The effect appears to be enhanced by a low dietary intake of potassium containing fluids. Randomized controlled trials in hypertension patients indicate that reducing sodium intake by 80-100 mM (4.7 – 5.8gms) / day from an initial intake of around 180 mM (10.5gm) per day will reduce blood pressure by an average of around 4-6 mm Hg systolic (Burt et al., 1995). It has been found that black, obese and elderly subjects are most sensitive to changes in dietary salt. The aim of dietary sodium reduction should be to achieve an intake of less than 6 gms per day of sodium chloride or less than 100 mmol (15.8 gms) per day of sodium. Patients should be advised to avoid added salt and processed foods.
5. Complex dietary changes

Vegetarians have lower blood pressure than meat eaters (Rouse et al., 1983) and vegetarian dietary patterns can lower blood pressure in hypertensive patients. In a recent study it has been found that by increasing fruit and vegetable consumption caused blood pressure to fall by 3/1 mm hg while the added measure of reducing fat intake led to a fall of 6/3 mm hg (Appel et al., 1997).

Hypertensive patients should be advised to eat more fruit and vegetable, to eat more fish and to reduce their fat intake.

6. Increased Physical Activity

Sedentary patients should be advised to take up modest level of aerobic exercise on a regular basis, such as brisk walk or a swim for 30-45 min 3-4 times a week. This form for exercise is better than more strenous forms and may lower systolic pressure by about 4-8 mm hg. Isometric excesses like weight lifting have a pressor effect and should be avoided.

7. Psychological Factors and Stress

Helping individuals to cope with stress may have an important impact on their blood pressure and on compliance with anti-hypertensive medication. Whether there are more direct effects of sustained stress on long term blood pressure levels is a subject of on going research. To data, trials of various stress management procedures for blood pressure control have been unconvincing.
Other life style measures with limited or unproven efficacy in lowering blood pressure include biofeed back, micronutrient alterations and dietary supplementation with calcium, magnesium and factors.

II. Drug Treatment For Lowering Blood Pressure

The six main drug classes used world wide for blood pressure lowering treatment are: diuretics, beta blockers, calcium antagonists, ACE inhibitors, angiotensin II receptor antagonists and alpha adrenergic blockers.

There are important differences between these drug classes on their effects on morbidity and mortality. While there is a large body of data demonstrating the benefits of the older agents such as diuretics and beta blockers, there are fewer data available about calcium antagonists and ACE inhibitors and no reliable data available about alpha blockers or the most recent classes of agents such as angiotension II receptor antagonists (Hansson, 1995).

<table>
<thead>
<tr>
<th>Class of Drug</th>
<th>Compelling indications</th>
<th>Possible indications</th>
<th>Compelling contraindications</th>
<th>Possible contraindications</th>
</tr>
</thead>
<tbody>
<tr>
<td>Diuretics</td>
<td>Heart failure</td>
<td>Diabetes</td>
<td>Gout</td>
<td>Dyslipidaemia</td>
</tr>
<tr>
<td></td>
<td>Elderly patients</td>
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<td></td>
<td></td>
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<tr>
<td></td>
<td>Systolic hypertension</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Beta-Blockers</td>
<td>Angina</td>
<td>Heart failure</td>
<td>Asthma and chronic</td>
<td>Dyslipidaemia</td>
</tr>
<tr>
<td></td>
<td>After myocardial</td>
<td></td>
<td>obstructive pulmonary</td>
<td>Athletes and physically</td>
</tr>
<tr>
<td></td>
<td>infarction</td>
<td></td>
<td>disease</td>
<td>active patients</td>
</tr>
<tr>
<td></td>
<td>Tachyarrhythmias</td>
<td></td>
<td>Heart block$^a$</td>
<td>Peripheral vascular</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>disease</td>
</tr>
<tr>
<td>ACE inhibitors</td>
<td>Heart failure</td>
<td></td>
<td>Pregnancy Hyperkalaemia</td>
<td>Bilateral renal artery</td>
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<td></td>
<td>Left ventricular</td>
<td></td>
<td></td>
<td>stenosis</td>
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<td></td>
<td>dysfunction</td>
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<td></td>
<td>After myocardial</td>
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<td></td>
<td>infarct</td>
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<tr>
<td></td>
<td>Diabetic nephropathy</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Calcium</td>
<td>Angina</td>
<td></td>
<td>Heart block$^b$</td>
<td>Congestive heart failure</td>
</tr>
<tr>
<td>Antagonists</td>
<td>Elderly patients</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Systolic hypertension</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Alpha-Blocker</td>
<td>Prostatic hypertrophy</td>
<td></td>
<td>Glucose intolerance</td>
<td>Orthostatic hypotension</td>
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<tr>
<td></td>
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<td></td>
<td>Dyslipidaemia</td>
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<tr>
<td>Angiotensin</td>
<td>ACE Inhibitor cough</td>
<td>Heart Failure</td>
<td>Pregnancy Bilateral renal</td>
<td></td>
</tr>
<tr>
<td>II Antagonists</td>
<td></td>
<td></td>
<td>Hyperkalaemia</td>
<td></td>
</tr>
</tbody>
</table>

$^a$Grade 2 or 3 atrioventricular block

$^b$Grade 2 or 3 atrioventricular block with verapamil or diltiazem

$^c$Verapamil or diltiazem
Principles Of Drug Treatment

1. Use of low doses of drugs to initiate therapy, beginning with the lowest available dose, in an effort to reduce adverse effects. If the pressure is short of adequate control with low dose of a single drug it is reasonable to increase the dose of the same drug, provided that it has been well tolerated.

2. Use of appropriate drug combinations to maximise hypotensive efficacy while minimising side effects. It is often preferable to add a small dose of second drug rather than increasing the dose of original drug. This way both the drugs are used in low range and are likely to be free from side effects. Fixed dose combinations are being increasingly made available in US and Europe.

3. Changing to a different class of drug altogether if there is very little response or poor tolerability to the first drug used, before increasing the dose of the first drug or adding a second drug.

4. Use of long acting drugs providing 24 hr efficacy on a once daily basis. The advantages of such drugs include improvement in adherence to therapy and minimisation of blood pressure variability as a consequence of smoother, more consistent blood pressure control this may provide greater protection against the risk of major cardiovascular events and development of target organ damage. (Conway et al., 1988; Mancia and Parati, 2000).
Initiation of drug Treatment

a. High/Very high Risk Groups:

For patients in the high and very high risk groups drug treatment should be instituted within a few days as even as repeated measurements have confirmed the patient's blood pressure. (Hansson, 1988)

b. Medium Risk Group Patients

1. Life style measures should be reinforced for at least 3 months before considering drug treatment.

2. If goal blood pressures are not attained within a maximum of 6 months, drug treatment should be initiated.

c. Low Risk Group (Grade 1 hypertension)

1. Life style measures should be used assiduously for 6 months.

2. If goal blood pressure are not attained within a maximum period of 6 months, drug treatment should be instituted within 1 year.

d. Borderline Patients (With DBP between 90mmhg and 94 mmhg SBP between 140 and 149 mm hg)

Doctor in consultation with the patient may choose to resort to lifestyle measures alone to lower the pressure and reduce cardiovascular risk.

e. High Normal Blood Pressure [(130-139/85-89 mg) with diabetes mellitus and/or renal insufficiency]
CHOICE OF ANTIHYPERTENSIVE DRUGS

All available drug classes are suitable for initiation and maintenance of anti-hypertensive therapy, but the choice of drugs is influenced by many factors including:

1. Socio-economic factors that determine drug availability in different population groups.

2. The cardiovascular risk factor profile of the individual patient.

3. The presence of target organ damage, of clinical cardiovascular disease, renal disease and diabetes.

4. The presence of other co-existing disorders that may either favour or limit the use of particular classes of anti hypertensive drugs.

5. Variation is individual patient responses to drugs from different classes.

6. The possibility of interactions with drugs used for other conditions present in the patient.

7. The strength of the evidence for reduction of cardiovascular risk within the drug class is question.

The physician should tailor the choice of drug to the individual patient, after taking all those factors together with patient preference. (Hedner, 2000)

1. Diuretics: They constitute one of the most valuable classes of anti hypertensive drugs. They are inexpensive, effective, well tolerated
in low doses and have been clearly shown to prevent major cardiovascular events including stroke and CHD. Most of the unwanted side effects of diuretics such as potassium depletion, reduced glucose tolerance, ventricular ectopic beats and impotence are associated with the use of high dose. There is some evidence from observational studies that the risk of sudden cardiac death in patients treated with non-potassium sparing diuretics can be reduced by their combination with potassium sparing diuretics (Cruickshank et al, 1987; Siscovick et al, 1994).

Diuretics should be used in low doses, in order to reduce the adverse effects while still reaping the benefits. Particularly recommended for treatment of elderly patients with systolic hypertension.

2. Beta blockers: They are safe, cheap and effective for use as monotherapy or in combination with diuretics, dihydropyridine calcium antagonists and alpha blockers. Heart failure used to be a clear contra-indication to the use of beta blockers in standard doses. However there is emerging evidence that they may have a beneficial effect when used in very low starting doses in some patients with heart failure (Doughty et al., 1997). They should be avoided in patients with obstructive airway disease and peripheral vascular disease.
3. **ACE inhibitors:** They are safe and effective in lowering blood pressure and are much less expensive now than when first introduced. They are particularly effective in reducing morbidity and mortality in heart failure and in retarding the progression of renal disease in patients with insulin dependent diabetics mellitus, especially in presence of proteinuria (Lewis et al., 1993; Garg et al., 1995). Their most common adverse effect is dry cough and their most serious adverse effect is very rare but life threatening angioedema.

4. **Calcium Antagonists:** They are effective and well tolerated in lowering blood pressure. Long acting calcium antagonists are preferred and rapid onset short acting formulations should be avoided. They are particularly recommended for elderly patients with systolic hypertension (Staessen et al., 1997).

   Adverse effects include tachycardia, flushing, ankle edema and constipation.

5. **Angiotensin II receptor antagonists:** They are the latest major group of anti-hypertensive to become generally available, and are of particular value in patients with heart failure. There is still no reliable evidence of their effects on cardiovascular risk in patients with hypertension.

   However they have few side effects, which may encourage adherence to therapy and appear to offer one advantage over
ACFi inhibitors i.e. a near absence of cough as a side effect (Timmermans et al., 1991b).

6. Alpha Blockers: They are safe and effective in lowering blood pressure. There is still no evidence about their effects on cardiovascular risk in hypertensive subjects. Their main side effect is postural hypotension, which may be a particular problem in elderly patients. Assessment of standing blood pressure is essential. These drugs may have advantages in subjects with dyslipidaemia or glucose intolerance or prostatic enlargement (Singh and Goyal, 1999).

NEWER DRUGS

1. Renin Inhibitor (Enalkiren)

Four different types of renin inhibitors have been produced which include renin antibodies, analogue to pro-segment of prorenin, analogue of pepstatin and angiotensinogen analogue. Of these, only angiotensinogen analogue has a potential for therapeutic use. It competes with angiotensinogen for binding to active sites of renin but these are not cleaved by renin. Enalkiren competitively inhibits action of renin and prevents conversion of angiotensinogen to angiotensin I. IV infusions of enalkiren in dose of 0.03, 0.1, 0.3 and mg/kg or patients with mild to moderate hypertension, produced a fall in systolic and diastolic BP at all doses. (Frishman et al., 1994; Wood et al., 1994).
2. Potassium channel openers (Pinacidil)

It is a new class of anti hypertensive drug. In vascular smooth muscles, increased outward $K^+$ current causes cellular hyperpolarisation and decreased $Ca^{2+}$ entry into the cell resulting in vasorelaxation. Pinacidil is a new class of $K^+$ channel activator which is rapidly absorbed from GI tract with a half life of 3 hrs.

It has potential in the treatment of mild to moderate hypertension, and a possible role in hypertension secondary to renal disease but its overall place in the therapy of hypertension remains to be clarified.

Combination with thiazide diuretic is preferable. Its initial dose is 12.5 mg twice daily and maintenance dose of 25mg twice daily. Its side effects include fluid retention, edema, palpitations, flushing, nasal congestion and postural hypotension. (Friedel et al., 1990; Kato, 1993).

3. Neutral Endopeptidase Inhibitor (Candoxatil)

Neutral endopeptidases are responsible for metabolism of atrial natriuretic factor (ANF).

Blockade of neutral endopeptidase by drugs like landoxatril leads to raised levels of ANF which in turn produces natriuresis and suppression of renin-angiotensin-aldosterone system and leads to a fall in B.P. (Singh and Goyal, 1999).
2. Dopamine (D_1-receptor) Agonist (Fenoldopam)

Fenoldopam is a selective agonist of D_1 receptor which are present in renal, mesenteric, coronary and other blood vessels. Dopamine agonists thus produce selective vasodilatation of splanchnic and renal arterial bed. It has a very rapid onset short duration of action and has been successfully used by intravenous infusion for the treatment of hypertensive emergency. A comparative trial of fenoldopam and sodium nitroprusside has shown fenoldopam to have better renal hemodynamic response, as it improves renal perfusion, improves GFR and increases sodium excretion. (Weber, 1988).

3. Serotonin Antagonist (ketanserin)

Ketanserin selectively blocks 5-HT_{2A} receptors and \( \alpha_1 \) adrenergic receptors which is responsible for its hypotensive effect.

It also reduces platelet aggregation. Oral bio-availability is about 50% with a half life of 20-30 hours oral preparation is available in many European countries for treatment of systemic hypertension (Brogden et al., 1990).

4. Dual adrenergic \( \alpha_1 \) and calcium channel blockers (monatepil)

It is a new imidazole derivative. Apart from reducing blood pressure, it also lowers serum cholesterol and has antilipid peroxidation properties. Therefore it is considered to be a potential antiatherosclerotic agent.
5. Dual ACE and Neutral Endopeptidase Inhibitors (alatriopril)

They are "broad spectrum antihypertensive agents" effective in majority of hypertensive patients. ACE inhibitors are effective in high renin and normal-renin hypertension and much less effective in hypertension with volume expansion and suppressed renin levels. Volume expansion is a potent stimulus for cardiac secretion of atrial natriuretic peptide (ANP) inhibition of neutral endopeptidase will lower BP in volume-expanded hypertension. (Wilkins et al., 1993; Bralet et al., 1994).
MONOTHERAPY VERSUS COMBINATION THERAPY

Drug Monotherapy

When drugs from the main classes available are used as monotherapy at the recommended doses, they produce very similar blood pressure reductions. In general, the sizes of the blood pressure reductions increase with the initial level of blood pressure, but typically the placebo-adjusted reductions average about 4-8% for both systolic and diastolic blood pressure. Thus for patients with blood pressures of about 160/95 mm Hg, the usual reduction produced by monotherapy would be about 7-13 mm Hg systolic and 4-8 mm Hg diastolic.

Clearly for many patients with hypertension such reductions in blood pressure would not restore optimal or even non-hypertensive blood pressure levels. (Zanchetti, 2000).

Drug Combination Therapy

Combination therapy of several of the available drug classes has been shown to produce blood pressure reductions that are more than those produced by any group of individual agents used alone. The HOT study, in which blood pressure was lowered to below 90mm Hg in over 90% of patients, demonstrated that combination therapy was necessary in 70% of participants. Combinations with fully additive hypotensive effects will deliver blood pressure reductions that are around twice as low as those obtained with a single drug, of the order
of 8-15% or 12-22 mm Hg systolic and 7-14 mm Hg diastolic for patients with blood pressures of 160/95 mm Hg. (Mancia and Grassi, 1996; Hansson et al., 1998).

Effective Drug Combinations (JNC VI 1997)

1. Diuretic and beta-blocker

2. Diuretic and ACE inhibitor (or All receptor antagonist)

3. Calcium antagonist (DHP) and beta-blocker

4. Calcium antagonist (DHP) and ACE inhibitor

5. Alpha blocker and beta blocker.

Effective drug combinations utilise drugs from different classes in order to obtain the additive hypotensive effect that comes from combining drugs with different primary actions, while minimising the compensations that limit the fall in blood pressure. Combinations of limited value generally result from combining drugs that work through similar mechanisms so that their hypotensive actions may be less than additive, or drugs that have similar side effects so that the risk of adverse effect is increased (Menard, 1993).

Status of the combination under Evaluation:

A fixed dose combination of losartan 50 mg and amlodipine 5 mg is available in India under the brand name of Losar-A marketed by
UNICHEM Laboratories, Mumbai. It is claimed to be a powerful combination with smooth sustained 24 hr, BP control. It is indicated for mild to moderate hypertensives specially diabetic hypertensive. The most important benefit of this combination is its effectiveness in a single daily dose.
ANGIOTENSIN II RECEPTOR ANTAGONISTS

The renin-angiotensin system is integral to the mechanisms that sustain hypertension and also has a pivotal role in determining the prognosis of this condition. (Gibbon, 1998)

Perhaps the single most interesting observations came from the work of Laragh and his colleagues (Brunner et al., 1993), who noted that hypertensive patients with inappropriately high renin levels appeared to be at increased risk of stroke and heart attack.

This finding emphasizes the need for anti hypertensives that not only reduce BP but also have inhibitory effects on the renin-angiotensin system. (Laragh, 1992)

Angiotensin II is known to be a powerful vaso-constrictor with a key role in sustaining high blood pressure levels in hypertension Renal pathology, particularly that connected with the structure and function of the glomerulus is also directly affected by angiotensin II.

Angiotensin II has powerful growth effects both in the myocardium and the arterial wall and there is evidence that angiotensin II induces left ventricular hypertrophy (Weber, 1997).

I. Discovery of Angiotensin II Receptor Antagonists

The actions of Angiotensin II are mediated by specific receptors located on various target organs (e.g. Adrenal cortex, kidney arterioles, sympathetic nerve endings).
Pharmacological inhibition of RAS started in 1971 with saralasin, the first specific peptide antagonists of Ang II.

Although saralasin reduced arterial pressure in hypertensive patients with high renin levels its therapeutic potential was limited, because this peptide antagonists has a very short half life, is not orally bioavailable and still possesses significant agonistic properties.

In 1980s two patents were granted to Furakawa and Colleagues at Takeda chemical industries (Osaka, Japan) for simple N-benzyl-imidazoles such as S-8307 and S8308 which were characterized to be very weak but selective nonpeptide angiotensin II receptor antagonists with a competitive mode of action but were orally inactive.

Oral activity was obtained for biphenyl carboxylic acid derivatives EXP 7711 and EXP 9654 (Timmermans et al., 1991a).

A further advance in the design of non-peptide Angiotensin II receptor antagonist was provided by DUP 753 which was found to competitively antagonize Ang II induced responses in various in vitro and in vivo preparations with marked anti hypertensive effects in spontaneously hypertensive rats.
The enzymatic cascade within the renin-angiotensin system produces Ang II as the main key effector.

Ang II is involved in activating a cluster of secondary mechanisms, converging upon a single homeostatic mechanism, the preservation of a preset minimal level of arterial pressure. This target is served both by direct (vaso pressor), delayed (sodium and fluid retention) and long term (vascular restructuring and growth) mechanisms (Menard, 1993; Kristina et al., 1999).
All these actions of Ang II are mediated through its binding to specific receptors in the plasma membrane of target organ cells.

ANGIOTENSIN II Receptors: Radioligand receptor binding studies have revealed the existence of two main subtypes of Ang II receptors AT\(_1\) and AT\(_2\). (Timmermans et al., 1993; DeGaspero et al., 1995; Goodfriend and Elliot, 1996) Both subtypes seem to have a lot in common i.e. they have analogous geometry, in that both contain polypeptide chains with 360 amino acids in the shape of seven transmembrane domains connected with intracellular G proteins but there is only 30% sequence homology.

AT\(_1\) Receptor: AT\(_1\) receptor is genetically linked to chromosome 3 and is ubiquitously present in vascular tissue. It has unique receptiveness to Ang II antagonist losartan. (Dzau, et al., 1993)

AT\(_1\) receptor has now been unequivocally established as the main mediator of the effects of Ang II on blood pressure levels and cardiovascular growth (Goodfriend et al., 1996).

AT\(_2\) receptors: AT\(_2\) receptor gene is located on the 'x' chromosome (Koike et al., 1994)

AT\(_2\) receptor is contained within selective areas in the brain and the kidney. AT\(_2\) receptor can be selectively blocked by PD123319 (Timmermans et al., 1993). The role of AT\(_2\) receptor has remained open to speculation so far.
III. Mechanism of Ang II Blockade by Non-Peptide AT₁ Receptor Blockers

Losartan is the prototype drug representing the sartan family. It is the main pharmacological tool to date.

Sartans interact with amino acids in the transmembrane domains of AT₁ receptors and occupy space among the seven helices, preventing the binding of Ang II. The sartan-receptor complex is not internalized and hence does not influence the receptor population on the cell surface (Goodfriend and Elliot, 1996).

Inove et al developed a series of hypothetical models of antagonist-receptor interaction which show that EXP 3892 and candesartan are insurmountable AT₁ antagonists while Losartan is a surmountable antagonist. (Vanderhayden et al., 1999).

IV. Pharmacological Studies with Ang II receptor antagonists

Most clinically significant effects of Ang II are attributed to the angiotensin type I receptor. Losartan is the prototype of this class of drugs. They undergo first pass metabolism in the liver by a cytochrome P450 mechanism. With the exception of candesartan, other approved sartans do not require biotransformation in order to display their full effect. (Timmermans et al., 1990; Birkenhager et al., 1999).

Sartans are eliminated through variable proportion of urinary and biliary excretion. The effect of all All antagonists was assessed in normotensive volunteers by monitoring their inhibitory effect on the BP response to
exogenous angiotensin (Christen et al., 1991). A linear relationship between drug dose, plasma levels of the drug (or the metabolites) and the pressor inhibitory effects was found for all drugs. Total inhibition of the pressor response to exogenous AII was demonstrated only with Irbesartan at a dose of 300mg. There was no effect on heart rate and no interference with mechanisms contributing to the response of arterial pressure to orthostatism.

A reactive rise in blood levels of renin and AII was observed following administration of all AIIA’s. Repeated administration of AIIA’s was associated with both a progressive and mild increase in plasma levels of renin and AII measured before drug intake and a tendency for the inhibitory pressor effect to be greater on the eighth than on the first day (Delcretaz et al., 1995). Whether unopposed stimulation of AT$_2$ receptors may mediate a depressor response to AII remains to be established in humans (Mimran et al., 1999).

Only a minimal or no effect of AII receptor blockade was observed on plasma aldosterone under basal conditions but inhibition of aldosterone response to AII was documented (Ogihara et al., 1995).

In normal man, the vasoconstrictor effect of AII on the forearm vasculature was inhibited by 44% and 66% within 4 to 6 hrs after a single oral administration of 20 and 100 mg losartan respectively. 10 mg dose of enalapril was found to potentiate bradykinin induced forearm dilatation (Cockroft et al., 1993).
Assessing the dose-related efficacy of anti-hypertensive drugs is a complex issue. Most programmes undertaken to assess the clinical efficacy and safety of presently approved AIIA's have included large numbers of patients with mild to moderate (sometimes severe), mostly essential hypertension in well controlled, parallel group studies of 8-12 wk duration (Menard et al., 1997; Sever, 2000).

Yet the minimally active and usual doses were not always well defined. With regards to losartan, the threshold dose could be in the range of 10-25 mg, whereas 50, 100 and 150 mg of losartan O.D. Were found to be equipotent (Grandman et al., 1995).

Thus, the regulatory agencies considered once a day administration of 50 mg losartan as the usual dose although further studies suggested that 50 mg bid was more effective but as effective as 100 mg O.D. (Weber et al., 1995).

Reeves et al., (1998) suggested that the anti-hypertensive effect of irbesartan may increase with increasing doses before reaching a plateau at 300 mg. No such analysis is available for other AIIA's. It is of interest that for several reasons, including the lack of fully convincing data, recommended dosages and practical rules for titration vary quite markedly between countries. (Mimran et al., 1999).

A few comparative studies between AIIA's were conducted. Over 8 wks, candesartan 16 mg was significantly more effective than losartan 50 mg
Tolerability and side Effects

AIIA's appeared "neutral" with regards to metabolic parameters. In patients with essential hypertension, insulin sensitivity was evaluated by euglycaemic hyperinsulinemic clamp technique was not affected by chronic administration of losartan (50-100 mg od for 4-12 wks) (Laako et al., 1996). However, treatment by candesartan (8 mg/day for 2 wks) resulted in a significant improvement in insulin sensitivity, similar to that achieved by the ACE Inhibitor lisinopril.

Cough is by far the most common side effect associated with the use of almost all ACEIs with an incidence estimated at 5 to 20%. Cough can develop within 1 week to 6 months after the start of therapy and may be more common in women (Israeli, 1992). The advent of AllA's provided an alternative to ACEI when blockade of the renin-angiotensin system is effective.

Angioneurotic edema occurs in 0.1% to 0.2% of ACE I treated patient and usually within hours to 1 week after the start of ACEIs. This complication was reported in a few occasional subjects treated by All A's, namely in patients with previous occurrence of ACE I-related angioedema (Dzielak, 1998).

This suggests that AllA's should not be substituted to ACEIs in patients with a history of idiopathic and ACE I-induced angioedema.
Dysgeusia has been reported in a patient treated with valsartan (Heeringa, 1998).

VII. Angiotensin Antagonists in Renovascular Disease

In a double-blind cross over study the effect of a 4 day period of treatment by valsartan (80 mg OD) in 12 patients with unilateral renovascular disease (7 with fibromuscular dysplasia and 5 with atheromatous stenosis) was assessed. Whereas a significant fall in arterial pressure was observed, no change in renal function was detected. (Magri et al., 1999).

The effect of acute administration of 50mg captopril and 200 mg losartan on renal function was assessed in the same 17 patients with atheromatous renal artery stenosis and moderate renal failure, and overall decrease in GFR was observed. Interestingly, transient anemia occurred after both agents in a patient with bilateral renal artery stenosis (Mimran et al., 1998).

Renal effects of Angiotensin Antagonists

No significant change in renal plasma flow or glomerulus filtration rate was observed after acute administration of losartan at doses of 50 to 100 mg. In contrast, acute administration of eprosartan resulted in renal vaso dilatation (Price et al., 1997).

Losartan as well as Irbesartan had a natriuretic effect, which was sustained after chronic treatment.

In hypertensive patients with renal impairment, candesartan (8mg daily for 5 days) exerted a renal dilatory effect and a fall in filtration fraction similar
Losartan. Such an effect could be a predictor of a favourable influence on the evolution of renal function with time in patients with chronic renal failure.

A striking uricosuric effect of losartan was demonstrated by mother compound instead of active metabolite E-3174. In contrast no uricosuric effect of the other presently available AIIA's such as irbesartan, eprosartan and candesartan was found.

Cardiac Effects of Angiotensin II Antagonists

Regression of left ventricular hypertrophy may be an important requirement for the use of antihypertensive agents. Only recently, two studies conducted in small no. of patients, showed that losartan treatment was associated with a reduction in left ventricular mass more marked than that achieved during treatment by verapamil 240 mg OD or hydrochlorothiazide (Timmermans et al., 1991b; Datilof, 1993).

LOSARTAN

Losartan potassium (DuP 753 or MK-954) is an orally active, non-peptide angiotensin II (Ang.II) receptor antagonist. It is the first of a new class of drugs to be introduced for clinical use in hypertension.

This novel agent binds competitively and selectively to the Ang.II subtype I (AT\(_1\)) receptor thereby blocking Ang.II-induced physiological effects (Siegl, 1993).
PHARMACODYNAMIC PROPERTIES

1. Inhibition of Angiotensin II (All) Activity

1. Inhibition of Receptor Binding.

Losartan Potassium is highly and specifically bound to AT\(_1\) receptors. It is 10,000 times more selective for the AT\(_1\) than the AT\(_2\) receptor (Abdel Rahman et al., 1992; Goa and Wagstaff 1996).

In rats losartan potassium 10mg/kg/day significantly reduced the density of AT receptor from baseline in liver, kidney and adrenal cortex but not in adrenal medulla where AT\(_2\) receptors predominate.

2. Functional Antagonism of Ang.II Activity.

(a) In vitro and in Vivo –

Binding of losartan potassium to the AT\(_1\) receptor is saturable, reversible and competitive. In concentrations of 10\(^{-8}\) to 10\(^{-7}\) mol/l it caused parallel shifts to the right of the concentration contractile response or pressor response curve to Ang.II. The drug competitively blocked Ang.II induced contraction of rabbit aorta, guinea pig ileum and rat uterus and Ang.II induced pressor response in conscious or spinally pithed rats. In none of these test systems did the drug display any Ang.II agonist effects (Goa and Wagstaff, 1996).

(b) In Healthy Volunteers

Several studies have confirmed that losartan was well tolerated in healthy male normal subjects on a normal sodium intake. Losartan was very well
tolerated with no specific adverse effects or complaints different from placebo. (McIntyre et al, 1997) In early studies, there appears to be little effect of losartan on blood pressure up to 120 mg up to 10 days daily administration. As the renin angiotensin system in normal volunteers is not activated, these findings are not surprising.

There is no effect of losartan on heart rate in healthy subjects (Christen et al., 1991) and no interference with homoestatic mechanisms. Extensive formal investigations of sympathetic and parasympathetic autonomic reflexes revealed no evidence of an effect of losartan, and plasma and urinary catecholamines were not affected in normals (Christen et al., 1991). There is only limited information on intravenous administration of losartan or its metabolite E-3174. The highest doses of intravenous losartan (40 mg) and intravenous E-3174 (40 and 80 mg) studied caused modest fall in blood pressure in salt replete healthy subjects. (McIntyre et al, 1997). Losartan effects occurred with a delay probably related to the formation of the metabolite. These observations are consistent with the greater potency of the metabolite and the likelihood that metabolism of losartan to E-3174 is an important determinant of hemodynamic responses.

In all studies in humans with either oral or intravenous dosing, there was no indication of a partial agonist effect on angiotensin II receptors with either losartan or E-3174.

Although there is no evidence of a marked fall in blood pressure, in salt replete healthy subjects, a peripheral vasodilator action can be inferred from the blockade of pressor and localised venoconstrictor effects of angiotensin I and II infusions(McIntyre et al., 1997).
In single oral doses of 20 and 100mg, losartan potassium blocked the vaso-constrictor response to exogenous AngI and AngII in healthy individuals, as measured by forearm blood flow and changes in dorsal hand vein diameter (Cockroft et al., 1993). Similarly, the pressor response to exogenous AngI and AngII was inhibited by up to 95% in a dose-related fashion by single and multiple oral doses of losartan potassium 10 to 120mg. With doses 40mg and higher this effect persisted for at least 24 hrs (Weber, 1992).

Activation of renin-angiotensin system by disease, diuretics or salt depletion enhances the hemodynamic effects of losartan. In salt depleted normals, a long-lasting dose related fall in supine and standing systolic and diastolic blood pressure after losartan (10-100 mg) is unmasked by a low sodium diet plus frusemide (Doig et al., 1995). In this study, moderate salt depletion was achieved with frusemide and low salt diet (40 m Eq./day).

In normal volunteers, losartan was found to increase PRA and angiotensin II concentrations, however plasma aldosterone levels tend to fall (McIntyre et al., 1997) Renal effects of losartan have been studied in normal subjects. Losartan show a mild natriuretic effect and acute and transient uricosuric effect (McIntyre et al., 1997).

3. Effect on the RAS

Effects of losartan potassium on the RAS are consistent with inhibition of Ang.II activity. In healthy volunteers, losartan ≤ 100mg in single or multiple doses increased plasma renin activity and plasma AngII levels
but produced inconsistent effects on plasma aldosterone levels, compared with placebo. (Chirsten et al., 1991; Doig et al., 1993).

4. Hemodynamic and Cardiovascular Effects

Losartan potassium has been investigated both as monotherapy and in combination with hydro-chlorothiazide in randomized double blind multicentric clinical trials, usually of 8-12wks duration, involving a total of approximately 3700 patients. All comparative investigations included a placebo washout or active control run-in period and a placebo or active control during the main part of the study.

Placebo-adjusted trough to peak ratios in patient with hypertension were calculated as 60% for 50mg dose, 72% for the 100mg dose and 62-85% for losartan potassium 50mg plus hydrochlorothiazide 6.25 mg to 12.5mg (Ikeda et al., 1997). A ratio of ≥ 50% is considered indicative of a duration of activity permitting once-daily dosages. (Ruilope et al., 1996)

This aside, the effects of losartan potassium on blood pressure have been shown to extend throughout a 24-hour period. 24 hr ambulatory blood pressure monitoring in 14 patients given losartan potassium 50 to 100mg for 12 weeks demonstrated mean DBP decreases of 8 mm Hg during the day (0700 to 1900hrs) and 6.8 mm Hg at night (1900 to 0700hrs)(Byyny, 1996).

Studies in elderly patients have shown losartan 50 or 100mg/day to have an equivalent anti-hypertensive effectiveness to felodipine ER5 or 10mg/day and nifedipine gastrointestinal therapeutic system (GITS) 30-90mg/day over 12 weeks and to enalapril 10mg/day over 24 weeks
(Simpson et al., 2000). Vast majority of studies using animal models have demonstrated either a preventive or a regressive effect of losartan potassium against cardiac hypertrophy.

In patients with hypertension, losartan significantly reduced left ventricular mass index in randomized double blind trials (Crozier, 1995; Tedesco et al., 1998).

Results of ELITE I (Evaluation of Losartan in the elderly) study suggest that treatment with losartan reduced end-diastolic and end-systolic volumes. Aging, particularly in hypertensive individuals is often associated with impaired baro-receptor sensitivity. Like ACE inhibitors, losartan has been shown to improve baro-receptor function (Simpson et al., 2000).

Unlike ACE inhibitors, which decrease haemoglobin levels and blood viscosity, losartan appears to have no effect on haemotology (haemoglobin and plasma erythropoetin levels) and haemorheology. Losartan 50mg/day had no effect on QT dispersion in patients with heart failure. (Brooksby et al., 1998)

5. Effects on Renal Hemodynamics and Function

Renal function is preserved during losartan potassium administration. Glomerular filtration rate, renal blood flow, urine volume were unchanged in healthy volunteers following a single 100mg dose and in patients with hypertension given losartan potassium 50mg daily for periods of 7 days to one year (Goa and Wagstaff, 1996).

Losartan causes an acute and transient increase in uric acid excretion.
The uricosuric effect is associated with a modest decrease in serum uric acid. Losartan is known to decrease proteinuria (an albumin sparing effect) associated with renal failure or type 2 (NIDDM) diabetes mellitus.

6. Effects on Bradykinin

Losartan potassium did not affect forearm vasodilatation induced by exogenous bradykinin infusion in healthy volunteers. Because losartan potassium does not inhibit ACE, it would not be expected to produce elevated levels of bradykinin which are also implicated in ACE inhibitor-induced cough. (Cockcroft et al., 1993).

7. Metabolic and Neuroendocrine Effects

Serum levels of lipids or lipoproteins have remained unchanged during losartan potassium treatment for 4 weeks in patients with mild hypertension.

II. Pharmacokinetic Properties

Hepatic oxidation of losartan potassium yields pharmacologically active carboxylic acid metabolite E3174 (Stearns et al., 1995).
<table>
<thead>
<tr>
<th>Parameters</th>
<th>Losartan Potassium</th>
<th>E3174</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cmax (ug/l)</td>
<td>0.29</td>
<td>0.25</td>
</tr>
<tr>
<td>Tmax (h)</td>
<td>1.0hrs</td>
<td>4.1hrs</td>
</tr>
<tr>
<td>AUC_{0-\infty}(ug/L.h)</td>
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<td>1.9</td>
</tr>
<tr>
<td>T\textsubscript{1/2} (h)</td>
<td>2.1</td>
<td>6.4</td>
</tr>
<tr>
<td>CL\textsubscript{r}(L/R)</td>
<td>4.3</td>
<td>1.6</td>
</tr>
<tr>
<td>V\textsubscript{d}(L)</td>
<td>34</td>
<td>12</td>
</tr>
</tbody>
</table>

Oral bioavailability of losartan potassium is approximately 33% because of first pass metabolism and is largely unaffected by food.

Both compounds (losartan and E3174) are > 98% plasma protein bound (Weber et al., 1995).

Losartan was undetectable in plasma at 10 hours post dose whereas E3174 was measurable at 24 hrs. About 14% of the dose is converted to its active metabolite and less than 5% of a losartan potassium dose is excreted unchanged renally in patients with normal renal function. (Lom et al., 1998)

Clinically relevant effect of renal impairment on the pharmacokinetics of losartan are minimal. This has been confirmed in patients with varying degree of renal insufficiency given losartan. But patients with suspected renal artery stenosis should avoid losartan.
On the other hand, in patients with mild to moderate alcoholic cirrhosis plasma concentration of losartan potassium and E-3174 increased 5-fold and 17-fold respectively, oral bioavailability was doubled and total plasma clearance was halved. Dosage adjustment is therefore required in this population.

Drug Interactions

Losartan did not affect the pharmacokinetic properties of single dose warfarin or digoxin or hydrochlorothiazide.

The cytochrome P450 (CYP) enzymes CYP 2CP (major enzyme) and CYP3A4 are largely responsible for the biotransformation of losartan to E3174. Therefore drugs which affect these enzymes, such as cimetidine, fluconazole, itraconazole, ketoconazole, erythromycin phenobarbital and rifampicin have potential to affect pharmacokinetics of losartan (Simpson et al., 2000).

Therapeutic Efficacy

1. Hypertension

In the general population the anti-hypertensive efficacy of losartan 50mg or 100mg once daily was similar to that of other standard anti-hypertensive agents including enalapril 10 or 20mg, atenolol 50 or 100mg and felodipine extended release (ER) 5 or 10mg once daily, in trials of upto 12 weeks duration. (Shobha et al., 2000). The addition of hydrochlorothiazide produced additional decrease in BP. There was no alteration in the response to losartan in patients with renal failure or type
Diabetes mellitus. Losartan potassium appears beneficial in elderly patients, as evidenced by its similar efficacy to felodipine ER in a large trial. (Tsunoda et al., 1993; Farsang et al., 1999)

Losartan had a more favourable effect on quality of life than enalapril in elderly hypertensives. QOL was assessed by the general well being, physical symptom distress index-B, social participation and work performance and satisfaction evaluation tests (Burrel, 1997). Dizziness was the only drug-related adverse event reported more frequently with losartan potassium than with placebo. First dose hypotension has seldom been reported. There have been rare instances of angioedema, severe migrain and reversible ageusia developing during losartan potassium therapy (Goa and Wagstaff, 1996).

Hyperkalemia occurred in a small number of patients (1.5%). There have been transient increases in liver enzyme levels in a small number of patients taking losartan (Nygaard et al., 1996). Sudden withdrawal of losartan did not cause rebound hypertension.

The incidence of cough in double blind clinical trials was 3.1% with losartan.

Dosage and Administration

The recommended starting and maintenance dosage of losartan is 50mg once daily in patients with essential hypertension. In patients not responding to losartan monotherapy a stepwise titration may be used to once daily losartan 50mg in fixed combination with hydrochlorthiazide.
12.5mg and, if required, once daily losartan 100mg plus hydrochlorothiazide 25mg.

The maximum antihypertensive effect can be expected 3 to 6 weeks after initiation of losartan therapy (Lacourciere et al., 1999). Losartan may be given with or without food and with other antihypertensive agents. No dosage adjustment is required in the elderly. Losartan should not be given to pregnant women because of the risk of fetal and neonatal morbidity and mortality associated with drugs that act directly on the RAS as they can affect fetal renal perfusion. (McIntyre et al., 1997).

2. Heart Failure

Losartan Heart Failure (ELITE I) and Losartan Heart Failure survival (ELITE II) studies have examined the benefits of therapy with losartan in patients with heart failure.

Reports indicate improvements in exercise tolerance after treatment with losartan. Losartan recipients had a 32% lower risk for the secondary endpoints death or admission to hospital for heart failure due to a significant reduction in the risk of total mortality and fewer sudden cardiac deaths (Cuspidi et al., 1998). One possible explanation for the decrease in sudden deaths in losartan-treated patients is the absence of an effect on QT dispersion (Simpson et al., 2000).

Short term studies have indicated that the addition of losartan to an ACE inhibitors may provide additional benefits in patients with refractory heart failure not sufficiently controlled by their current regimen (Mallion et al., 1995).
Losartan was well tolerated in patients with heart failure. For patients with heart failure a starting dosage of losartan 12.5mg once daily is recommended by the manufacturer. The dosage should be titrated at weekly intervals to a maintenance dosage of 50mg once daily, as tolerated by the patient (Simpson, 2000).

The effects of losartan in patients with myocardial infarction are being evaluated in the ongoing randomized, controlled OPTIMAAL study. This trial is designed to evaluate the effects of losartan ≤ 50mg/day on mortality in patients with evidence of heart failure or left ventricular dysfunction after an acute myocardial infarction.

The RENAAL study compares losartan 50mg/day with optimal conventional anti-hypertensive therapy in patients with type 2 diabetes Mellitus and severe diabetic nephropathy. This study is presently underway with 1520 patients enrolled so far.

Losartan is effective for the treatment of hypertension with an excellent tolerability profile in man. It has been approved for marketing in several European countries and United States for the treatment of hypertension and represents the first new antihypertensive agent in over a decade.

The US patent covering losartan and EXP3174 was filed in 1988 and was issued in August 1992 to Dupont. This series of molecules has been widely used by many investigators in the world as a research tool for physiological studies of Angiotensin II.
This invention attracted the attention from Merck Research Laboratories, which resulted in a collaborative agreement to co-develop losartan. This has subsequently led to the formation of a new company, the Dupont Merck Pharmaceutical Company (Wilmington, DE, USA) in 1991. Thus losartan is also an agent that has founded a new company. (Wong et al., 1996).
CALCIUM CHANNEL BLOCKERS

Calcium ions are ubiquitous in mammalian biological processes, and are essential to the homeostasis of the entire cardiovascular system. For over a century it has been known that Ca\(^{2+}\) plays a central role in cardiac pacemaker activity and excitation-contraction coupling. The involvement of Ca\(^{2+}\) in these physiological processes means that functions such as myocardial contractility, myocardial oxygen consumption, ejection fraction, systolic wall pressure, arterial tone, peripheral vascular resistance, preload and afterload are all calcium dependent. In addition, coronary arterial tone is dependent on intracellular and extracellular Ca\(^{2+}\) concentrations.

For 50 years or more, drugs that are capable of regulating transmembrane ion flux have been investigated in an attempt to modify Ca\(^{2+}\) homeostasis for therapeutic application. In this respect, the calcium channel blockers represent the most successful attempt at pharmacological modification Ca\(^{2+}\) regulation and these drugs are now widely used in the management of hypertension and ischaemic heart disease.

As long ago as 1962, it was demonstrated that intravenous administration of verapamil lowered blood pressure in patients with hypertension, but not in subjects who were normotensive (Gonzalo et al., 1994). It was 20 years later, however before clinical utility of CCBs in hypertension was fully recognised.
Classification of CCBs (Luscher et al., 1993)

<table>
<thead>
<tr>
<th>Structure</th>
<th>Examples</th>
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<tr>
<td>Phenylalkylamines</td>
<td>Verapamil, Gallopamil</td>
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<tr>
<td>Benzothiazepines</td>
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<td>Dihydropyridines</td>
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<td></td>
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<td></td>
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<tr>
<td></td>
<td>Nisoldipine, Isradipine,</td>
</tr>
<tr>
<td></td>
<td>Nifedipine</td>
</tr>
</tbody>
</table>

Mode of Action of CCBs

The intracellular concentration of Ca^{2+} depends on two processes: release from sarcoplasmic reticulum and influx, which may occur via voltage-dependent L type (long acting) channels, receptor-operated channels and Na^{+} - Ca^{2+} exchangers. The CCBs selectively block the influx of Ca^{2+} through the L type channels, but do not affect the sarcoplasmic reticulum, mitochondrion (another intracellular Ca^{2+} store) or Na^{+} - Ca^{2+} exchangers.

Inhibition of L type channels causes a decrease in the intracellular Ca^{2+} concentration which through inhibition of Ca^{2+} mediated signalling causes muscle relaxation, only in tissues that are sensitive to CCB's namely smooth muscle and cardiac myocytes (Hering, 1998).
Pharmacokinetic Characteristics of CCBs in clinical use (Van Zwieten et al., 1996)

<table>
<thead>
<tr>
<th>Compound</th>
<th>Bio-availability %</th>
<th>Tmax</th>
<th>Plasma protein binding%</th>
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</tbody>
</table>

$T_{\text{max}}$ = Maximum effect; $F$ = fraction excreted without renal biotransformation; IR = immediate release; CC = coat core.

1 Immediate-release formulation
2 Controlled release formulation
Degree of tissue selectivity of CCB in clinical use

<table>
<thead>
<tr>
<th></th>
<th>Myocardium</th>
<th>Vessels</th>
<th>Sino-atrial node</th>
</tr>
</thead>
<tbody>
<tr>
<td>Verapamil</td>
<td>+</td>
<td>+</td>
<td>+</td>
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<tr>
<td>Diltiliazem</td>
<td>+</td>
<td>+</td>
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<tr>
<td>Nifedipine</td>
<td>+</td>
<td>++</td>
<td>-</td>
</tr>
<tr>
<td>Nimodipine(^1)</td>
<td>+</td>
<td>+++</td>
<td>-</td>
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<tr>
<td>Felodipine(^2)</td>
<td>+</td>
<td>++++</td>
<td>-</td>
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<tr>
<td>Nisoldipine(^2)</td>
<td>+</td>
<td>++++</td>
<td>-</td>
</tr>
<tr>
<td>Amlodipine</td>
<td>+</td>
<td>++++</td>
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</tbody>
</table>

+ = Selectivity; - = no selectivity

1 Cerebral vessels

2 Coronary vessels
AMLODIPINE

Amlodipine is a dihydropyridine calcium antagonist which was synthesized by scientists at Pfizer central research and has FDA approved labeling for once daily administration for hypertension, chronic stable angina and vasospastic angina.

Pharmacology

Amlodipine occupies the plasma membrane dihydropyridine receptor and causes competitive blockade of the voltage-operated slow calcium channels and thereby impedes the transmembrane influx of calcium ions into vascular smooth muscle cells and cardiac muscle cells. (Gonzalo et al., 1994).

It is more selective for vascular smooth muscle than for cardiac muscle. Amlodipine reduces peripheral vascular resistance without affecting cardiac conduction or cardiac contractility unlike other dihydropyridines it has a slow rate of onset of effect with maximum smooth muscle relaxation compared to other dihydropyridines. (Vetrovec, 1989).

In humans the dominant effects of amlodipine are consequent on vasodilatation in doses of upto 10 mg administrated to normotensives. Daily doses of 5-25 mg produce dose related decreases in the systemic blood pressure in hypertensive patients and the duration of effect exceeded 24 hours. Amlodipine lowers peripheral vascular resistance without causing reflex tachycardia.
Amlodipine, has been found to be effective in once daily dosage in the control of hypertension with falls in both systolic and diastolic blood pressures (Hoggs, 1989).

In addition, renal vasodilatation is also seen with amlodipine therapy and results in modest increase in GFR. Amlodipine reduces myocardial $O_2$ requirement and also causes vasodilatation of the coronary vascular bed and due to these effects it has been found to cause a dose-related increase in duration of exercise before the onset of angina in patients with coronary artery disease. No effect of amlodipine has been noted on renin-angiotensin system or on the sympathetic nervous system. Amlodipine does not affect atrial natriuretic factor concentration in humans.

In Healthy Volunteers:

Following once daily oral administration, amlodipine is virtually completely absorbed from the intestinal tract. Peak plasma levels are reached after 6 to 12 hours. It has a relatively high bioavailability of 60 to 80%. Steady state plasma concentrations in healthy volunteers are achieved after the 7th dose, without accumulation since this substance follows linear pharmacokinetics.

Amlodipine undergoes slow hepatic metabolism. The metabolites possess no calcium antagonistic properties and are excreted via urine and in faeces (Steffen, 1999).

Studies in normotensives have shown that amlodipine heightens the sympathetic alpha-adrenoceptor activity with an increase in
peripheral resistance in the early morning hours. (Panza and Epstein, 1991).

However, amlodipine does not lead to a stimulation of noradrenaline and adrenalin secretion at rest or during exercise (Steffen, 1999).

One earlier report of amlodipine indicates that single dose administration of 5 mg had no effect on resting heart rate and mean arterial pressure (Goldsmith, 1995).

However, in doses upto 10 mg administered to normotensives, vasodilatory effects of amlodipine are evident (Hoggs, 1989).

In a recent study by Lay et al. (2001) effect of amlodipine on cardiopulmonary performance in volunteers was assessed. It was a double-blind cross-over study of amlodipine (10 mg daily for 2 weeks) on oxygen uptake and catecholamine responses during exercise in eight volunteers. Despite a therapeutic plasma concentration of amlodipine (22.8 +/- 9 ng/ml) there was no change in resting heart rate or blood pressure. Amlodipine did not cause a significant change in oxygen uptake at the anaerobic threshold or at maximum exercise and there was also no change in heart rate or catecholamine responses. Although there was an awareness of peripheral vasodilatation and reports of lethargy during active treatment period, the volunteer had no objective evidence of a decrease in cardiopulmonary performance.

The plasma elimination half-life of amlodipine after a single dose is 36 hours but increases upto 45 hours after reported administration.
with steady-state plasma concentrations reached after seven days of therapy (Faulkner et al., 1986).

In elderly patients and in patients with hepatic cirrhosis, the elimination half-life is increased to 48 and 60 hours respectively after the administration of a single dose. This property of long duration of action allows amlodipine to be administrated once a day. It appears that no adjustment in dosage is necessary for patients with renal insufficiency. (Frick et al., 1988).

Monotherapy

Amlodipine as a single agent in patients with mild to moderate hypertension has shown efficacy comparable to those of atenolol, captopril, hydrochlorothiazide, verapamil and felodipine.

Broadhurst et al., (1992) evaluated the efficacy of amlodipine 5-10 mg with 24-hour ambulatory intra-arterial blood pressure monitoring in hypertensive patients. Amlodipine significantly reduced blood pressure over the 24 hour period without affecting the heart rate or the normal circadian variation in blood pressure.

Webster et al (1988) found that amlodipine 10-25 mg/day reduced both SBP and DBP during an eight week treatment period. Mroczek et al (1998) confirmed these findings by 24 hour ambulatory blood pressure monitoring.

Velasco et al (1991) studied amlodipine 2.5-10 mg once daily versus captopril 25-mg once daily in hypertensive patients. After two weeks
both agents were found to be equally effective in reducing SBP and DBP. Further more 24-hour blood pressure monitoring demonstrated that amlodipine produced a more sustained and uniform reduction in blood pressure than captopril, whose antihypertensive effect started to diminish after nine hours.

The most convincing data supporting the use of amlodipine in the treatment of hypertension came from treatment of mild hypertension study. In this multicentric study more than 800 patients were studied. The effects of non-pharmacological therapy in combination with placebo or one of the following antihypertensive drugs. Amlodipine 5 mg/day, Acebutolol 400 mg / day, doxazosin 2 mg / day, chlorthalidone 15 mg / day, enalapril 5 mg / day were evaluated over a period of 4.4 years.

Amlodipine, like other agents produced a better blood pressure reduction and better control than placebo. No significant differences in blood pressure reduction were noted among the groups that received other drug (Gonzalo et al., 1994).

Combination therapy

Amlodipine has also been studied in combination with other agents. Amlodipine plus captopril reduced SBP and DBP significantly more than placebo plus captopril. Similarly amlodipine (2.5-10 mg / day) with hydrochlorothiazide 50mg/day had a significantly larger antihypertensive effect than hydrochlorothiazide plus placebo.
Amlodipine can be indicated as first or second line agent for hypertension (Gonzalo et al., 1994) and as per American JNC VI reports (1997) and WHO:ISH (1999) guidelines, amlodipine has the therapeutic status of first line agent.

Effect on serum glucose and lipids

Amlodipine has been found to increase the high density lipoprotein cholesterol to total cholesterol ratio and reduce serum triglyceride level (Gonzalo et al., 1994).

QUALITY OF LIFE

Along with safety and efficacy, well being and the quality of life need to be considered when selecting antihypertensive drugs. Two clinical trials have evaluated amlodipine impact on the quality of life. The treatment of mild hypertension study examined the effect of five antihypertensives on seven quality-of-life indicators (general health, energy/fatigue, mental health, general functioning satisfaction with physical abilities, social functional and social contacts). Amlodipine had a beneficial effect on all seven indicators (Gonzalo et al., 1994).

ADVERSE EFFECTS

As with other calcium antagonist drugs, peripheral edema and skin erythema occur in 5-10% and facial flushing in 2-5% of patients. Complaints of fatigue and somnolence occurred more frequently in amlodipine treated group (Mancia et al., 1996).
Gingival hypertrophy and gynaecomastia has been attributed to amlodipine therapy.

Dose-related peripheral edema is also a commonly reported adverse effect, it does not appear to be related to sodium or water retention and is probably caused by an increase in capillary pressure and filtration and is not responsive to diuretic therapy.

**DRUG INTERACTIONS**

Calcium antagonists are frequently prescribed with digoxin in patients with multiple cardiovascular problems. Unlike other calcium antagonists amlodipine has not been shown to alter pharmacokinetics of digoxin in healthy volunteers (Gonzalo et al., 1994).

Unpublished reports have indicated that there is no interaction between amlodipine and cimetidine and amlodipine and warfarin in healthy volunteers. Food does not alter rate and extent of absorption of amlodipine.

Thus we can see that both angiotensin II receptor antagonist losartan and DHP calcium antagonist amlodipine are long acting drugs with trough-peak ratios of more than 50%. Also, they are metabolically neutral and do not have significant adverse effects. Therefore once a day administration of a fixed dose combination of amlodipine and losartan was chosen for the present study.
SUBJECTS, MATERIALS & METHODS
The study was conducted at the Ranbaxy clinical Pharmacology unit, II floor, Majeedia Hospital, Jamia Hamdard, New Delhi. ICH:GCP Guidelines(1996) and all the relevant standard operating procedures (SOPs) of the Ranbaxy CPU were followed while conducting the study.

STUDY DESIGN

A double blind, comparative, parallel study to evaluate the hemodynamic changes and adverse event profile of once daily administered combination of amlodipine and losartan was planned. The study was carried out in 24 volunteers and one patient with mild hypertension. Subjects were divided into two groups and each group after a placebo run-in period of one week received either amlodipine or losartan and a placebo for the initial two weeks. In the following two weeks placebo was replaced with the other active drug. Subjects underwent various evaluation tests at the allotted time points during the course of the study as described below.

STUDY POPULATION

The study was conducted in healthy, normo-tensive males. One female patient with mild hypertension who volunteered to participate in the study was also enrolled.

SELECTION OF SUBJECTS

Inclusion Criteria

1. Were in the age range of 18-45 years.
2. Were neither underweight nor overweight for his height as per the stipulation of life insurance corporation of India Height. Weight chart for non-medical cases.

3. Had given written informed consent to participate in this study.

4. Be of normal health as per determined by medical history and examination of the subjects performed within 15 days prior to the commencement of the study.

5. The following lab determinations were performed for:

i. Presence or absence of disease markers of HIV, Hepatitis B virus and syphilitic infections.

ii. Levels of haemoglobin, total leucocyte count, differential count and erythrocyte sedimentation rate.

iii. Values of serum creatinine, serum aspartate amino transferase, serum alkaline phosphatase, serum bilirubin, fasting plasma glucose and serum cholesterol.

iv. Chemical and microscopic examination of urine.

Exclusion Criteria

1. History of allergy to amlodipine or losartan.

2. Any evidence of organ dysfunction or any clinically significant deviations from the normal in physical and clinical determinations.

3. Physical handicap or disability
4. History of serious head injury or neurological disorder.

5. History of psychiatric illness which may impair the ability to provide written informed consent.

6. Regular smoker more than 20 cigarettes per day and have difficulty in abstaining from smoking during the study period.

7. Use of enzyme modifying drug within 30 days of any systemic medication (including OTC) within 14 days prior to the day 1 of the study.

8. Participation in any clinical trial within 6 weeks preceding the day of the study.

SELECTION OF PATIENTS

Inclusion criteria

1. Ambulatory patients of either sex

2. Age 30–75 years

3. Mild to moderate uncomplicated essential hypertension with sitting diastolic blood pressure between 90-114 mm of hg, both values inclusive (Korotkoff's phase V) after 1 week of placebo run in period (JNC VI).

4. Patients may be either newly diagnosed hypertensives, previously diagnosed but currently not under anti-hypertensive drug therapy or patients in whom a change of current anti-hypertensive drug
therapy is planned either due to lack of adequate response, adverse effects or for reason of dosage convenience.

Exclusion criteria

1. Patients with severe (sitting DBP 114 mm Hg.), accelerated or malignant hypertension.

2. Patients with secondary hypertension

3. Patients with history of MI, cardiac surgery or any other cardiac intervention or stroke in past 3 months.

4. Patients with severe heart failure, renal artery stenosis (Unilateral/Bilateral)

5. Patients in whom treatment with ACE inhibitors or angiotensin receptor antagonists in the past has been associated with oliguria and/or progressive azotaemia.

6. Patients receiving potassium containing medications or potassium supplements or medications which may interfere with the actions of the study drugs (other anti-hypertensives including diuretics, NSAIDS).

RANDOMIZATION

The randomization schedule was generated using the SAS statistical software.
BLINDING PROCEDURE

The randomisation schedule was kept with the supervisor who deputed a colleague. The colleague did dispensation of the medication throughout the study period. Subjects were unaware as to which drug they were receiving. The observer too was unaware as to which medication a given subject had been taking. Single blind procedure was followed with the female hypertensive patient.

NUMBER OF SUBJECTS

Thirty six adult healthy volunteers, seemingly likely to meet the requirements of this study, underwent study specific screening. Twenty four of these who met all the requisite criteria entered the study. Twelve subjects were allotted to each treatment group as per the randomization schedule.

STUDY MEDICATIONS

Amlodipine

Amlodipine 2.5mg tablets (Batch No: SM (1074) 004, Expiry date May 2002) containing amlodipine salt equivalent to 2.5 mg of amlodipine manufactured by Ranbaxy Research Labs Limited India. Dose of amlodipine for healthy volunteers was 5 mg while dose of amlodipine used for hypertensive patient was 2.5 mg.
Losartan

Losartas 50 mg tablets (Batch No. A005, Expiry date July 2002) containing losartan equivalent to 50 mg of losartan manufactured by Intas Pharmaceutical Ltd. India. Dose of losartan used in healthy volunteers was 100 mg while in the hypertensive patient 50 mg dose was administered.

DRUG PRODUCT RECEIPT, HANDLING AND ACCOUNTABILITY

The drug products were received by the Clinical Pharmacologist designate at the Ranbaxy Clinical Pharmacology Unit (CPU) and were logged-in. These were stored under prescribed storage conditions in a controlled access area (drug store).

DRUG ADMINISTRATION

Subjects were randomly allocated to the treatment groups. For the first week, i.e. week 1, the subjects received one drug-placebo in the form of a capsule (The placebo was so chosen as to match with the formulation of the second active drug that was to be added on during the last two weeks of the trial). After this first week the subject received two formulations till the end of the study. For the next two weeks i.e. week 2 and 3 one active medication was added to the placebo. In the subsequent last two weeks of the trial i.e. week 4 and 5 the second active medication replaced placebo and the subjects received two active medication. The study drugs and placebo were stored in a container and each container was labelled so as to
comply with the legal labeling requirements for medicinal products used in clinical trials.

All study medications were stored in a secure place as per the storage conditions specified. A count of returned formulations was performed at each visit to the CPU. All unused study medication was returned to the Clinical Pharmacologist, at the end of the study.

ADMISSION AND STAY

The study did not require overnight stay of the subjects/patient in the ward.

STUDY PROCEDURES

Visit number 1 (on day-1)

Each subject/patient was informed about the purpose and requirements of the study and written informed consent was obtained.

Details of the subjects/patient’s medical history, physical examination, concomitant medication and bodyweight were recorded.

The subjects/patient underwent the following procedures:

Clinic Blood Pressure

Supine blood pressure was recorded after a 10 minute rest. The subject was then asked to stand up for 2 minutes and standing blood pressure was recorded. On both the occasions blood pressure recording was done using a mercury sphygmomanometer (Diamond) and the measurements were recorded to the nearest 2 mm Hg. Thus
readings were recorded with an interval of one minute between the readings and the average of the two readings was calculated. Systolic blood pressure was recorded and the value corresponding to the Korotkoff's phase I and diastolic to Phase V.

- The subject/patient was asked regarding the adverse events
- The subject/patient was trained for ambulatory blood pressure monitoring.
- The subject/patient was issued one week supply of medication (placebo).
- The subject/patient was instructed to report to the OPD after six days on day -6.

Visit Number 2 (Day-6)

Ambulatory Blood Pressure

- Ambulatory blood pressure was recorded using Space Labs instrument. The instrument was programmed to record blood pressure every fifteen minutes in the day time and every half an hour during the night time. Data was considered for analysis if at least 85% of the readings were within the acceptable range defined as

(a) pulse pressure > 15 and < 150 mm Hg

(b) Diastolic blood pressure > 40 mm and < 140 mm Hg
(c) Systolic blood pressure > 50 and < 245 mm Hg

Data for the whole 24 hour recording, daytime recordings and night time recordings were analysed separately. Mean SBP, DBP and HR were compared in all the three time periods. The percentage of recordings that were above the period limits and considered separately for the comparisons of both systolic and diastolic loads.

- The subject was instructed to come the next day.

Visit Number 3 (Day-7)

- Blood Pressure was taken as specified above and the following parameters were recorded.

Electrocardiogram

A 12 lead electrocardiogram was recorded with the subject lying down on a Portrait ECG machine. Changes in the RR, PR, QR, QRS and QT intervals were assessed. Further any deviation from the baseline record (in the form of appearance of ectopic beats etc.) was also recorded. Prolongation of the PR interval beyond 0.2 sec was considered as evidence of first-degree AV block.

Systolic time Intervals

The following three time intervals were measured using the 'Nihon Kohden' mechanocardiograph:

1. Electromechanical Systole (QS₁): The time from the onset of electrical activation of the left ventricle until the start of
relaxation. Time of onset of electrical activation of the left ventricle corresponds to the start of the 'q' wave in the lead II of the electrocardiogram and the timing of the aortic value closure to the aortic component of the second heart sound recorded by the phonocardiogram.

(II) **Left Ventricular Ejection Time:** This is the time interval during which blood is ejected into the aorta and corresponds to the interval between the start of the rapid upstroke till the dichrotic notch on the indirect carotid pulse tracing.

(III) **Pre-ejection period:** The interval between the onset of electromechanical systole and start of ejection. \( \text{PEP} = \text{QS}_2 - \text{LVET} \)

As heart rate is important determinant of the systolic time interval, correction procedure as described below were applied to express values as those projected to occur at zero heart rate.

\[
\text{Corrected QS}_2 = 2.1 \times \text{HR} + \text{QS}_2
\]

\[
\text{Corrected LVET} = 1.7 \times \text{HR} + \text{LVET}
\]

\[
\text{Corrected PEP} = 0.4 \times \text{HR} + \text{PEP}.
\]

These corrected values were used for statistical analysis and are depicted in the results.

**Blood Analysis**

7ml of venous blood for estimation of the following parameters was drawn before administration of the dose.
Serum uric acid

Haemoglobin

Haematocrit

Serum sodium

Serum potassium

Body weight was recorded using a weighing machine

- Pill counting was undertaken

- All spontaneously volunteered, observed and elicited adverse events were recorded.

- The subject/patient was issued the next week's drugs and instructed to report to the CPU after one week (Day 8).

C.P.U. Visit No. 4 (Day 8)

- Blood pressure recording was done as specified above

- Electro cardiogram was recorded as specified above.

- All spontaneously volunteered, observed and elicited adverse events were recorded.

- Body weight was recorded using a weighing machine.

- Pill counting was undertaken.
The patient/volunteer were issued drugs for next week and instructed to report to the CPU on day 15. The hypertensive patient was instructed to report on Day 14 to the CPU.

**CPU Visit (day 14)**

- Ambulatory blood pressure was recorded as specified above.
- The patient was instructed to report to the CPU on Day 15.

**C.P.U. Visit No. 5 (Day 15)**

- Blood pressure was recorded as specified above.
- ECG was recorded as specified above
- Systolic time interval were recorded as specified above
- Pill counting was undertaken.
- All spontaneously volunteered, observed and elicited adverse drug events were recorded.
- The subject/patient was issued the next 6 days supply of drug.
- The subject/patient was instructed to report to the CPU after one week.
- Blood sampling was done. Along with the parameters specified above, liver function tests were done in healthy volunteers.
C.P.U. Visit No. 6 (Day 22)

- Blood pressure was recorded as specified above. ECG was recorded as specified above.
- Pill counting was undertaken
- All spontaneously volunteered, observed and elicited adverse drug events were recorded.
- The subject/patient was issued the next week’s medication and administered medication for that day.
- The subject/patient was instructed to report to the CPU after 6 days.

C.P.U. Visit No. 7 (Day 28)

- Ambulatory blood pressure recording was done as specified above.
- The patient/volunteer were instructed to take the medication meant for that day and were asked to report to the unit the next day.

C.P.U. Visit No. 8 (Day 29)

- Blood pressure was recorded as specified above.
- ECG was recorded as specified above.
- Systolic time intervals were recorded as specified above.
- Body weight was recorded on weighing balance.
• All spontaneously volunteered, observed and elicited adverse drug event were recorded.

• Pill counting was done.

• Blood sampling was done.

Blood Sampling

A blood sample of 15 ml was collected during the enrolment for the lab determinations. For each patient/volunteer the total volume of blood withdrawn did not exceed 50 ml till the end of the study.

After collection, blood samples were centrifuged under refrigeration as soon as possible to separate serum.

Adverse Events

At each visit spontaneously volunteered, observed and elicited (by general questioning) adverse events were recorded. The subjects/patient were asked the following question at each visit "Have the medication upset you in any way since your last visit?"

All adverse events reported by subject/patient were recorded in the CRF with information about severity (i.e. Whether mild, moderate or severe), date of onset, duration and action taken regarding study drug.

In the event of a serious (i.e. Any experience which is fatal or life threatening, permanently disabling, requires hospitalization, is a cancer, overdose or congenital anomaly) or unexpected (i.e., not
previously expected in nature, severity or frequency) adverse event, whether or not it is thought to be related to the test substance, a provision of the investigator to inform the Chief Supervisor immediately and Jamia Hamdard Institutional Review Board within 24 hrs was enforced.

Compliance criteria

The subjects/Patient taking at least 20 of the twenty eight day medication was considered as compliant with the protocol.

Data recording and compilation

All data generated during the conduct of the study was directly entered in the case record forms as governed by the protocol, except the analytical data of the clinical laboratory which was transcribed into the case record forms and the raw data retained by the laboratory for records. All raw data and transcribed data forms, was completed by the investigator or an assistant, assisting in the study.

Restrictions

Volunteers/patient were instructed not to take any concomitant medications. In an event where he/she has to take any drug he/she was asked to report to the CPU and this was recorded and brought to the notice of the chief supervisor or the co-supervisor and their advise was adhered to.
Diet

All subjects were instructed to take standard food throughout the study period.

Activity

Subject / patient were instructed to follow their normal activities and not make sudden deviations from the routine.

ETHICAL CONSIDERATIONS

Basic Principles

This research was carried out as per the WHO guidelines for 'Good Clinical Practice (GCP for trials on Pharmaceutical Products; ICH (Step 5) 'Guidance for Good Clinical Practice and the principles enunciated in the Declaration of Helsinki (South Africa 1996).

Institutional Review Board

The protocol was reviewed and approved by the Jamia Hamdard Institutional Review Board. Letter from the Chairman, Jamia Hamdard Institutional Board approving the protocol is being appended.

Informed consent

The purpose of the study, the procedures to be carried out and the potential burdens and hazards were described to the subjects / patient in non-technical terms. Subjects/ patients were required to read, understand and sign a consent form summarising the discussion
prior to enrolment. A copy of the informed consent statement used is being appended.

Data Handling and statistical analysis

Data Handling: Entries in the case record forms were made with a black ball point pen. Correction of the entered data was made by crossing out the incorrect entries with a single line (so that original entries are visible). Erasure/ blocking out by any other method was not resorted to. The correct data was entered next to the crossed out data.

Statistical analysis

Statistical analysis was performed on data from twenty three subjects who completed the study.

Summary statistics mean ± SD (n) is presented. Within group comparison was computed by the Student's paired t- test. Between group comparison was done using Student's unpaired t-test. All tests were at the level of significance of 5%. 
RESULTS
Results are presented as mean ± standard deviation (SD). In order to avoid the difficulty in comparison due to the varying baseline in the different groups, the changes produced have also been presented as percentage of the baseline.

Within each treatment group, the end combination therapy values were compared with end monotherapy values by employing the Student’s paired t-test. The design of the study also provided an opportunity to have an insight into the effect, if any of the order in which the drugs were administered. With this in mind the changes effected by the two groups were compared employing the Students unpaired t-test.

A probability value of less than 0.05 was taken as the level of statistical significance.

All the subjects except one completed the study as per protocol. One subject in the losartan monotherapy followed by amlodipine add on group, was withdrawn one week after the start of the study on health grounds. He developed fever and malaise and was diagnosed as suffering from malaria. He could not be evaluated for any parameter, as he was withdrawn before completion of placebo-run-in period. All other subjects met the requisite compliance criteria. Results of the various hemodynamic tests are described in the following section. The next section contains the safety parameters such as weight record, laboratory parameters and adverse events record.
ARM A: Amlodipine monotherapy followed by losartan add on. This arm consisted of 12 subjects, who received amlodipine initially for two weeks and losartan and amlodipine combination for the subsequent two weeks. In addition one hypertensive patient was enrolled in this group.

I) CLINIC BLOOD PRESSURE

Supine Systolic

Two weeks of treatment with amlodipine decreased the mean systolic blood pressure by 1.6%. Addition of losartan complimented the action of amlodipine in that the fall in blood pressure increased to 2.2% (Table No. 1A, Fig No. 1).

Supine Diastolic

The fall in diastolic blood pressure observed was 0.69%, on treatment with amlodipine. The addition of the second drug losartan substantially increased the fall in blood pressure from 0.69% to 2.3% (Table No. 1A, Fig. No. 1).

Supine heart rate

Treatment with amlodipine produced a change in supine heart rate of +3.7%. Addition of losartan to amlodipine produced changes in heart rate of +4.8% (Table No. 1A).
Standing systolic

The fall in blood pressure with the subject standing was 0.8% with amlodipine. Addition of losartan increased the fall from 0.8% to 1.3%.

The fall in blood pressure was less in magnitude as compared to the reduction in the supine posture. Nevertheless it was not a significant fall (Table No. 1A, Fig. No. 2).

Standing diastolic

Amlodipine alone caused a fall of 1.4%. Addition of losartan to amlodipine increased the fall in pressure from 1.4% to 3.2% (Table No. 1A, Fig No. 2).

Standing Heart Rate

Treatment with amlodipine produced a change in standing heart rate of +3.6%. Addition of losartan to amlodipine produced change in heart rate of +5.2% (Table No. 1A, Fig. No. 2).

II) AMBULATORY BLOOD PRESSURE (ABP)

ABP was recorded at the end of placebo run-in period and at the end of treatment with the combination.

24. Hour recording

The number of systolic readings that were above the period limits in the entire day was decreased by 36.9% and so was the time period during which the systolic reading were above the period limits by 56%.
Diastolic load in terms of both the number of readings and the time period during which the diastolic readings were above the period limits was also similarly reduced by 64% and 58% respectively (Table No. 2A).

Mean SBP showed a fall of 3.7% and DBP 4.1%, both the changes were statistically significant (p < 0.05). There was a slight increase in the heart rate of 3%. There was a fall in mean arterial pressure by 3.6% (Table No. 2A, Fig. No. 3).

Day time recordings

Both, the number of systolic readings and the time period during which the readings were above the period limits were reduced by 81.8% and 80.5% respectively.

Diastolic readings above the period limits reduced by 74% and also the time period during which the reading were above the period limits, reduced by 68%. The day time systolic and diastolic blood pressure recording were hardly influenced by the administration of this combination. Mean SBP showed decrease by 3.9% while DBP fell by 4.9%. Both these changes were statistically significant (p < 0.05). The mean arterial pressure was reduced by 4.4% while the heart rate showed a slight decrease by 4.1%. (Table No. 3A, Fig. No. 4)
Night time recording

Both the number of systolic readings and the time period during which the readings were above the limit were decreased by 39% and 50.8% respectively.

Diastolic load in terms of both the number of readings and the time period during which the diastolic readings were above the period limits was also reduced, like the influence on the systolic readings, by 61% and 60% respectively. Despite the magnitude in the changes observed they failed to achieve statistical significance.

The mean SBP was decreased by 3.8% (p < 0.05) and DBP by 4.7%. Mean heart rate increased by 4.9% while mean arterial pressure reduced by 3.4% (Table No. 4A, Fig. No.5).

II). E.C.G.

RR Interval

Treatment with amlodipine showed a decrease in ventricular rate by 2%. The addition of losartan had little impact on the effect of amlodipine. (Table No. 5A, Fig. No.6).

PR Interval

Amlodipine caused a shortening of PR interval by 2.4%. the addition of losartan to amlodipine complimented the action of amlodipine, producing a decrease of 5.7%. However these changes were not significant statistically (Table No. 5A, Fig. No. 6).
QRS Duration

No significant effect was noticed in the treatment group. amlodipine almost had little impact (+0.30%) while addition of losartan produced an increase in QRS duration by 1%. (Table No. 5A, Fig. No.6).

QTc Interval

Amlodipine reduced the QTc interval by 3.7%. Addition of losartan resulted in a nett increase in the QTc interval by 6.2%. There was no statistically significant change. (Table No.5A, Fig. No. 6).

IV SYSTOLIC TIME INTERVALS

Electromechanical systole

Amlodipine per se increased the QS₂ index by 2.5% and this action was complimented by addition of losartan to 3.7% (Table No. 6A, Fig. No.7).

Left ventricular ejection time

Amlodipine had little impact on this parameter (0.59% increase) while addition of losartan further increased it to 0.92% in this parameter. (Table No. 6A, Fig. No. 7). The increase however was not statistically significant.

Pre-ejection period

Amlodipine decreased the index by 10.5% the addition of losartan countered this decrease. The nett decrease now was only 0.98%.
None of these changes were statistically significant. (Table No. 6A, Fig. No.7).

LVET / PEP Ratio

Amlodipine increased the ratio by 15.6%, addition of losartan countered this increase. The nett increase now was 3.1%. However these changes were not significant statistically.
<table>
<thead>
<tr>
<th>Group</th>
<th>B</th>
<th>A</th>
<th>A+L</th>
</tr>
</thead>
<tbody>
<tr>
<td>N</td>
<td>12</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Supine</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>SBP</td>
<td>115.3</td>
<td>113.4</td>
<td>112.2</td>
</tr>
<tr>
<td>±SD</td>
<td>8.21</td>
<td>4.34</td>
<td>5.4</td>
</tr>
<tr>
<td>Change #</td>
<td>-1.8 (1.6)</td>
<td>-3.1 (2.2)</td>
<td></td>
</tr>
<tr>
<td>DBP</td>
<td>71.8</td>
<td>71.3</td>
<td>70.1</td>
</tr>
<tr>
<td>±SD</td>
<td>6.6</td>
<td>7.5</td>
<td>4.6</td>
</tr>
<tr>
<td>Change #</td>
<td>-0.5 (0.69)</td>
<td>-1.7 (2.3)</td>
<td></td>
</tr>
<tr>
<td>HR</td>
<td>69.9</td>
<td>72.5</td>
<td>73.3</td>
</tr>
<tr>
<td>±SD</td>
<td>10.2</td>
<td>9.0</td>
<td>8.7</td>
</tr>
<tr>
<td>Change #</td>
<td>+2.6 (3.7)</td>
<td>+3.4 (4.8)</td>
<td></td>
</tr>
<tr>
<td>Standing</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>SBP</td>
<td>111.1</td>
<td>110.2</td>
<td>109.6</td>
</tr>
<tr>
<td>±SD</td>
<td>5.1</td>
<td>5.8</td>
<td>6.1</td>
</tr>
<tr>
<td>Change #</td>
<td>-0.9 (0.8)</td>
<td>-1.5 (1.3)</td>
<td></td>
</tr>
<tr>
<td>DBP</td>
<td>74.9</td>
<td>73.8</td>
<td>72.5</td>
</tr>
<tr>
<td>±SD</td>
<td>9.1</td>
<td>5.5</td>
<td>5.9</td>
</tr>
<tr>
<td>Change #</td>
<td>-1.1 (1.4)</td>
<td>-2.4 (3.2)</td>
<td></td>
</tr>
<tr>
<td>HR</td>
<td>72</td>
<td>74.6</td>
<td>75.8</td>
</tr>
<tr>
<td>±SD</td>
<td>7.5</td>
<td>8.2</td>
<td>5.8</td>
</tr>
<tr>
<td>Change #</td>
<td>+2.6 (3.6)</td>
<td>+3.8 (5.2)</td>
<td></td>
</tr>
</tbody>
</table>

Systolic Blood Pressure (SBP) Diastolic Blood Pressure (DBP) in mm of Hg; Heart rate in beats/min, B = Baseline. # Figures in Parenthesis = % change from basal value. P > .05 on comparison Between (A) and (A+L).
Fig. No. 1: SUPINE SYSTOLIC AND DIASTOLIC BLOOD PRESSURE: PERCENTAGE CHANGES ON TREATMENT WITH AMLODIPINE(A) AND LOSARTAN(L) AS COMPARED TO THE BASE LINE

![Bar chart showing percentage changes in systolic (SBP) and diastolic (DBP) blood pressure across different treatment groups.](image)
Fig. No. 2: STANDING SYSTOLIC AND DIASTOLIC BLOOD PRESSURE: PERCENTAGE CHANGES ON TREATMENT WITH AMLODIPINE(A) AND LOSARTAN(L) AS COMPARED TO THE BASE LINE
# AMBULATORY BLOOD PRESSURE

Table No. 2A: CHANGES ON TREATMENT WITH COMBINATION OF AMLODIPINE AND LOSARTAN, 24 HOUR RECORDINGS

<table>
<thead>
<tr>
<th>% Readings above period Limits</th>
<th>% of time readings were above period Limits</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Systolic</strong></td>
<td><strong>Diastolic</strong></td>
</tr>
<tr>
<td>B A+L</td>
<td>B A+L</td>
</tr>
<tr>
<td>6.3 2.3</td>
<td>5.6 2.0</td>
</tr>
<tr>
<td>Change -4 (36.9)</td>
<td>-3.8 (64)</td>
</tr>
<tr>
<td><strong>Systolic Blood pressure</strong></td>
<td><strong>Diastolic Blood Pressure</strong></td>
</tr>
<tr>
<td>B A+L</td>
<td>B A+L</td>
</tr>
<tr>
<td>115 110.7*</td>
<td>70.4 67.5*</td>
</tr>
<tr>
<td>Change# -4.3 (3.7)</td>
<td>-2.9 (4.1)</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Mean arterial pressure</th>
<th>Heart Rate</th>
</tr>
</thead>
<tbody>
<tr>
<td>B A+L</td>
<td>B A+L</td>
</tr>
<tr>
<td>85.7 82.6</td>
<td>78.9 81.3</td>
</tr>
<tr>
<td>Change#</td>
<td>-3.1 (3.6)</td>
</tr>
<tr>
<td></td>
<td>+ 2.4 (3.0)</td>
</tr>
</tbody>
</table>

# figures in Parenthesis = % Change from basal value
*P< 0.05 as compared with baseline record
FIG. NO. 3: 24 HOUR AMBULATORY BLOOD PRESSURE MONITORING: CHANGES IN SYSTOLIC AND DIASTOLIC BLOOD PRESSURE ON TREATMENT WITH COMBINATION OF AMLODIPINE AND LOSARTAN

Percentage

100 100

SBP DBP

B A+L

Treatment Groups

96.3 95.9
## AMBULATORY BLOOD PRESSURE

Table No. 3A: CHANGES ON TREATMENT WITH COMBINATION OF AMLODIPINE AND LOSARTAN: DAY TIME RECORDING

<table>
<thead>
<tr>
<th>% of reading above period limits</th>
<th>% of time the reading were above period limits</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Systolic</strong></td>
<td><strong>Diastolic</strong></td>
</tr>
<tr>
<td>B</td>
<td>B</td>
</tr>
<tr>
<td>3.8</td>
<td>0.69</td>
</tr>
<tr>
<td>Change</td>
<td>-3.11 (61.8)</td>
</tr>
<tr>
<td><strong>Diastolic</strong></td>
<td><strong>Systolic</strong></td>
</tr>
<tr>
<td>B</td>
<td>A+L</td>
</tr>
<tr>
<td>1.6</td>
<td>4.1</td>
</tr>
<tr>
<td>Change</td>
<td>-4.5 (74)</td>
</tr>
<tr>
<td><strong>Mean arterial pressure</strong></td>
<td><strong>Heart rate</strong></td>
</tr>
<tr>
<td>B</td>
<td>B</td>
</tr>
<tr>
<td>89.0</td>
<td>85.3</td>
</tr>
<tr>
<td>Change</td>
<td>-3.9 (4.4)</td>
</tr>
<tr>
<td><strong>A+L</strong></td>
<td><strong>A+L</strong></td>
</tr>
<tr>
<td>85.1</td>
<td>85.3</td>
</tr>
<tr>
<td>Change</td>
<td>+ 3.7 (4.1)</td>
</tr>
</tbody>
</table>

#Figures in parenthesis = % change from Basal value.

* P < 0.05 as compared with baseline record
Fig. No. 4: 24 HOUR AMBULATORY BLOOD PRESSURE MONITORING: DAY TIME CHANGES IN SYSTOLIC AND DIASTOLIC BLOOD PRESSURE ON TREATMENT WITH COMBINATION OF AMLODIPINE AND LOSARTAN
#AMBULATORY BLOOD PRESSURE#

##Table No. 4A: CHANGES ON TREATMENT WITH COMBINATION OF AMLODIPINE AND LOSARTAN: NIGHT TIME RECORDING##

<table>
<thead>
<tr>
<th>% of reading above period limits</th>
<th>% of time the reading were above period limits</th>
</tr>
</thead>
<tbody>
<tr>
<td>Systolic</td>
<td>Diastolic</td>
</tr>
<tr>
<td>B</td>
<td>A+L</td>
</tr>
<tr>
<td>13.6</td>
<td>8.3</td>
</tr>
<tr>
<td>Change #</td>
<td>-5.3 (39)</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Systolic Blood Pressure</th>
<th>Diastolic Blood Pressure</th>
<th>Mean arterial pressure</th>
<th>Heart rate</th>
</tr>
</thead>
<tbody>
<tr>
<td>B</td>
<td>A+L</td>
<td>B</td>
<td>A+L</td>
</tr>
<tr>
<td>107.6</td>
<td>*103.5</td>
<td>63.1</td>
<td>60.3</td>
</tr>
<tr>
<td>Change #</td>
<td>-4.1 (3.8)</td>
<td>-2.8 (4.7)</td>
<td>-2.6 (3.4)</td>
</tr>
</tbody>
</table>

#Figures in parenthesis = % change from Basal value.

* P < 0.05 as compared with baseline record
Fig. No. 5: 24 HOUR AMBULATORY BLOOD PRESSURE MONITORING: NIGHT TIME CHANGES IN SYSTOLIC AND DIASTOLIC BLOOD PRESSURE ON TREATMENT WITH COMBINATION OF AMLODIPINE AND LOSARTAN

![Bar graph showing the percentage of systolic (SBP) and diastolic (DBP) blood pressure changes in treatment groups B and A+L. The graph indicates a significant drop in blood pressure in the A+L group compared to the B group.]
**Fig. No. 5A: EFFECT OF AMLODIPINE (A) AND COMBINATION OF AMLODIPINE AND LOSARTAN (A+L) ON RR INTERVAL, PR INTERVAL, QRS DURATION AND QTC INTERVAL**

<table>
<thead>
<tr>
<th>Group</th>
<th>B</th>
<th>A</th>
<th>A + L</th>
</tr>
</thead>
<tbody>
<tr>
<td>N =</td>
<td>12</td>
<td></td>
<td></td>
</tr>
<tr>
<td>VR</td>
<td>79.4</td>
<td>77.8</td>
<td>78.1</td>
</tr>
<tr>
<td>±SD</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Change #</td>
<td>-1.6 (2.0)</td>
<td>-1.3 (1.6)</td>
<td></td>
</tr>
<tr>
<td>PR Interval</td>
<td>122</td>
<td>119</td>
<td>115</td>
</tr>
<tr>
<td>±SD</td>
<td>0.01</td>
<td>9.0</td>
<td>9.7</td>
</tr>
<tr>
<td>Change #</td>
<td>-3 (2.4)</td>
<td>-7 (5.7)</td>
<td></td>
</tr>
<tr>
<td>QRS Duration</td>
<td>65.9</td>
<td>66.1</td>
<td>66.6</td>
</tr>
<tr>
<td>± SD</td>
<td>0.01</td>
<td>0.01</td>
<td>0.01</td>
</tr>
<tr>
<td>Change #</td>
<td>+0.2 (0.30)</td>
<td>+0.7 (1.0)</td>
<td></td>
</tr>
<tr>
<td>QTc Interval</td>
<td>321</td>
<td>309</td>
<td>341</td>
</tr>
<tr>
<td>±SD</td>
<td>0.10</td>
<td>0.02</td>
<td>0.02</td>
</tr>
<tr>
<td>Change #</td>
<td>-12 (3.7)</td>
<td>+20 (6.2)</td>
<td></td>
</tr>
</tbody>
</table>

(PR Interval, QRS duration, QTc in milliseconds and ventricular rate (VR) in beats/min) B = Baseline

Ventricular rate calculated from mean RR interval # figures in Parenthesis = % change from the basal value
P < 0.05 on Comparison Between (A) and (A+L).
Fig. No. 6: ELECTROCARDIOGRAPHIC CHANGES IN PERCENTAGE ON TREATMENT WITH AMLODIPINE (A) AND LOSARTAN (L) AS COMPARED TO THE BASE LINE
# SYSTOLIC TIME INTERVALS

Table No. 6A: EFFECT OF AMLODIPINE (A) AND COMBINATION OF AMLODIPINE AND LOSARTAN (A+L) ON ELECTRO-MECHANICAL SYSTOLE, LEFT VENTRICULAR EJECTION TIME AND PRE-EJECTION PERIOD

<table>
<thead>
<tr>
<th>Group</th>
<th>B</th>
<th>A</th>
<th>A+L</th>
</tr>
</thead>
<tbody>
<tr>
<td>N =</td>
<td>12</td>
<td></td>
<td></td>
</tr>
<tr>
<td>QS₂ Index</td>
<td>516.8</td>
<td>529.6</td>
<td>535.9</td>
</tr>
<tr>
<td>± SD</td>
<td>14.8</td>
<td>10.2</td>
<td>20.0</td>
</tr>
<tr>
<td>Change #</td>
<td>+ 12.8 (2.5)</td>
<td>19.1 (3.7)</td>
<td></td>
</tr>
<tr>
<td>LVET Index</td>
<td>403.1</td>
<td>405.5</td>
<td>406.8</td>
</tr>
<tr>
<td>± SD</td>
<td>14.9</td>
<td>12.6</td>
<td>16.2</td>
</tr>
<tr>
<td>Change #</td>
<td>+2.4 (0.69)</td>
<td>+3.7 (0.92)</td>
<td></td>
</tr>
<tr>
<td>PEP index</td>
<td>129.2</td>
<td>115.6</td>
<td>127.9</td>
</tr>
<tr>
<td>± SD</td>
<td>11.1</td>
<td>16.0</td>
<td>21.8</td>
</tr>
<tr>
<td>Change #</td>
<td>-13.6 (10.5)</td>
<td>-1.3 (0.98)</td>
<td></td>
</tr>
<tr>
<td>LVET/PEP</td>
<td>0.32</td>
<td>0.37</td>
<td>0.33</td>
</tr>
<tr>
<td>± SD</td>
<td>0.03</td>
<td>0.11</td>
<td>0.06</td>
</tr>
<tr>
<td>Change # $</td>
<td>0.05 (15.6)</td>
<td>0.01 (3.1)</td>
<td></td>
</tr>
</tbody>
</table>

(QS₂ = Electromechanical systole, LVET = LEFT Ventricular Ejection time, PEP = Pre-Ejection period
LVET/PEP = LVET : PEP Ratio, B = Baseline)

# figures in parenthesis = % change from the basal value.

$ % change was always calculated before rounding off.

P < 0.05 on Comparison of (A) with (A+L)
Fig. No. 7: PERCENTAGE CHANGES IN SYSTOLIC TIME INTERVALS ON TREATMENT WITH AMLODIPINE(A) AND LOSARTAN(L) AS COMPARED TO THE BASE LINE.
ARM B: Losartan monotherapy followed by amlodipine add-on.

This arm consisted of a group of 12 subjects who received losartan initially for two weeks and losartan and amlodipine combination for the subsequent two weeks.

I) CLINIC BLOOD PRESSURE

Supine systolic

Two weeks of treatment with losartan decreased the mean systolic blood pressure by 1.1%. Addition of amlodipine complimented the action of losartan in that fall in blood pressure increased to 1.7%. But the difference was statistically not significant. (Table No. 1B, Fig. No. 8).

Supine diastolic

The fall in diastolic blood pressure observed was 1.7% on treatment with losartan. The addition of the second drug amlodipine increased the fall in blood pressure from 1.7% to 5.7%. None of these changes were significant statistically. (Table No. 1B, Fig. No.8).

Supine Heart Rate

Treatment with losartan produced a change in supine heart rate of +5.8%. The addition of amlodipine to losartan produced a change of +8.1%. Changes were statistically non-significant. (Table No.1B, Fig. No. 8).
Standing systolic

The fall in blood pressure with the subject standing was 1.3% with losartan. Addition of amlodipine increased the fall to 3.1%. The fall in blood pressure was less in magnitude as compared to the reduction in the supine posture. Never theless it was not a significant fall. (Table No. 1B, Fig. No.9).

Standing Diastolic

There was also a noticeable fall with losartan alone causing a fall of 3.6%. Addition of amlodipine to losartan increased the fall in blood pressure from 3.6% to 6.3%. However, these changes failed to achieve statistical significance. (Table no. 1B, Fig. No.9).

Standing Heart rate

Treatment with losartan produced a change in standing heart rate of 4.8%. Addition of amlodipine to losartan further increased it to 7.3%. Changes were statistically non significant. (Table No. 1B, Fig. No. 9).

II) AMBULATORY BLOOD PRESSURE (ABP)

ABP was recorded at the end of placebo run-in period and at the end of treatment with the combination.

24-hour recording

The number of systolic readings that were above the period limits in the entire day was decreased by 13.5% and the time period during which the systolic readings were above period limits reduced by 7.1%. These changes were not statistically significant.
Diastolic load in terms of both the number of readings and the time period during which the diastolic readings were above the period limits was also similarly reduced by 73% and 56.6% respectively. Despite the magnitude none of these changes were statistically significant.

Mean SBP showed a fall of 3.9% and mean DBP a fall of 7.9%. Both these observations were statistically significant (p < 0.05). Mean arterial pressure fell by 6.3% while mean heart rate showed a minor fall of 0.8%. Both these changes failed to achieve statistical significance. (Table No. 2B, Fig. No. 10).

DAY TIME RECORDINGS

The number of systolic readings which were above the period limits reduced by 68% unlike the influence on the number of times the systolic readings were above period limits which showed a change of +83.8%.

Mean SBP showed a decrease of 5.2% while mean DBP fell by 8.2%. Both these changes were statistically significant (p < 0.05). In terms of diastolic load, there was a decrease of 87.2% in number of readings above the period limits, and a decrease of 42% in the time period during which the readings were above the period limits. None of these changes were statistically significant.

The mean arterial pressure was reduced by 3.8%, and the heart rate was reduced by very little 1.7%. (Table No. 3B, Fig. No. 11).
Night time recordings

Both the number of systolic readings and the time period during which the readings were above the period limit were increased. In terms of percentage the former was increased by 40.2% and the latter by 20.5%

The number of diastolic readings and the time period during which the diastolic readings were above the period limits was also increased like the influence on systolic readings by 15% and 58.6% respectively. Despite the magnitude in the changes observed they failed to achieve statistical significance.

Mean SBP was decreased by 0.8% and DBP by 12.0%. The fall in DBP was statistically significant (p < 0.05). Moreover the increase in mean heart rate of 0.3% and the decrease in mean arterial pressure by 2.2% was not significant. (Table No. 4B, Fig. No. 12).

The combination thus exhibited efficacy both during the day time and during the night time.

III) E.C.G.

RR Interval

Treatment with losartan produced a decrease in ventricular rate by 2.3%. The addition of amlodipine had little further impact (2.9%) on the effect of losartan (Table No. 5B, Fig. No. 13).

PR Interval

Losartan produced an increase in PR interval by 16.2%. The addition of amloidine to losartan decreased it from 16.2 to 12.6%. None of
these changes were statistically significant (Table No. 5B, Fig No.13).

QRS Duration

No significant effect whatsoever was noticed in the treatment group. Losartan reduced the duration by 11.7%. The addition of amlodipine completely countered this effect of losartan and resulted in a nett increase of 2.6% (Table No. 5B, Fig. No. 13).

QTc Interval

Losartan increased it by 7.7%. Addition of amlodipine nearly completely countered this effect of losartan from 7.7% to 1% only. (Table no. 5B, Fig. No. 13).

IV) SYSTOLIC TIME INTERVALS

Electromechanical Systole

Losartan per se reduced the QS₂ index by 4.3% and this action was complimented by addition of amlodipine to 5.7%. (Table No. 6B, Fig. No. 14).

Left Ventricular Ejection Time

Losartan produced a change of -2.2% in this parameter while addition of amlodipine produced a change of -1.3%. (Table No.6B, Fig. No.14).

Pre-Ejection Period

Losartan reduced the index by 11.9% while addition of amlodipine reduced it by 9.4%.
None of these changes were statistically significant. (Table No. 6B, Fig. No. 14).

LVET/PEP Ratio

Losartan increase the ratio by 3%. Addition of amlodipine to losartan produced no change. (Table No. 6B, Fig. No. 14).
### TABLE NO. 1B: MEAN CLINIC BLOOD PRESSURE AND HEART RATE EFFECT OF LOSARTAN AND COMBINATION OF LOSARTAN AND AMLODIPINE

<table>
<thead>
<tr>
<th>GROUP</th>
<th>B</th>
<th>L</th>
<th>L+A</th>
</tr>
</thead>
<tbody>
<tr>
<td>N =</td>
<td>11</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Supine</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>SBP</td>
<td>116.7</td>
<td>115.4</td>
<td>114.6</td>
</tr>
<tr>
<td>±SD</td>
<td>4.8</td>
<td>4.6</td>
<td>4.0</td>
</tr>
<tr>
<td>Change #</td>
<td>-1.3 (1.1)</td>
<td>-2.1 (1.7)</td>
<td></td>
</tr>
<tr>
<td>DBP</td>
<td>75.1</td>
<td>73.8</td>
<td>70.8</td>
</tr>
<tr>
<td>±SD</td>
<td>4.9</td>
<td>7.3</td>
<td>5.7</td>
</tr>
<tr>
<td>Change #</td>
<td>-1.3 (1.7)</td>
<td>-4.3 (5.7)</td>
<td></td>
</tr>
<tr>
<td>HR</td>
<td>66.5</td>
<td>70.4</td>
<td>71.9</td>
</tr>
<tr>
<td>± SD</td>
<td>9.0</td>
<td>7.3</td>
<td>9.5</td>
</tr>
<tr>
<td>Change #</td>
<td>+3.9 (5.8)</td>
<td>+5.4 (8.1)</td>
<td></td>
</tr>
<tr>
<td>Standing</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>SBP</td>
<td>113.3</td>
<td>111.8</td>
<td>109.8</td>
</tr>
<tr>
<td>±SD</td>
<td>5.2</td>
<td>6.9</td>
<td>7.4</td>
</tr>
<tr>
<td>Change #</td>
<td>-1.5 (1.3)</td>
<td>-3.5 (3.1)</td>
<td></td>
</tr>
<tr>
<td>DBP</td>
<td>80.7</td>
<td>77.9</td>
<td>75.6</td>
</tr>
<tr>
<td>±SD</td>
<td>4.0</td>
<td>3.6</td>
<td>4.6</td>
</tr>
<tr>
<td>Change #</td>
<td>-2.8 (3.6)</td>
<td>-5.1 (8.3)</td>
<td></td>
</tr>
<tr>
<td>HR</td>
<td>74.6</td>
<td>78.2</td>
<td>80.1</td>
</tr>
<tr>
<td>± SD</td>
<td>9.2</td>
<td>8.5</td>
<td>7.7</td>
</tr>
<tr>
<td>Change #</td>
<td>+3.6 (4.8)</td>
<td>+5.5 (7.3)</td>
<td></td>
</tr>
</tbody>
</table>

Systolic blood pressure (SBP), Diastolic blood pressure (DBP) in mm of Hg Heart rate (HR) in beats/min; B = Baseline
# Figures in parenthesis = % change from basal value < 0.05 on Comparison between L and L+A (N = 12) Fig. No. 9: Standing systolic and diastolic blood pressure: percentage changes on treatment with Losartan(L) and Amlodipine(A) as compared to the base line
Fig. No. 8: SUPINE SYSTOLIC AND DIASTOLIC BLOOD PRESSURE: PERCENTAGE CHANGES ON TREATMENT WITH LOSARTAN (L) AND AMLODIPINE (A) AS COMPARED TO THE BASE LINE

![Bar chart showing percentage changes in blood pressure](chart.png)
Fig. No. 9: STANDING SYSTOLIC AND DIASTOLIC BLOOD PRESSURE: PERCENTAGE CHANGES ON TREATMENT WITH LOSARTAN(L) AND AMLODIPINE(A) AS COMPARED TO THE BASE LINE
AMBULATORY BLOOD PRESSURE

Table No. 2B: CHANGES ON TREATMENT WITH COMBINATION OF LOSARTAN AND AMLODIPINE 24 HOUR RECORDINGS

<table>
<thead>
<tr>
<th>% of reading above period limits</th>
<th>% of time the reading were above period limits</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Systolic</td>
</tr>
<tr>
<td></td>
<td>B</td>
</tr>
<tr>
<td>3.7</td>
<td>3.2</td>
</tr>
<tr>
<td>Change</td>
<td>-0.5 (13.5)</td>
</tr>
<tr>
<td>Systolic Blood Pressure</td>
<td>Diastolic Blood Pressure</td>
</tr>
<tr>
<td>B</td>
<td>A+L</td>
</tr>
<tr>
<td>113.9</td>
<td>109.5</td>
</tr>
<tr>
<td>Change #</td>
<td>-4.4 (3.9*)</td>
</tr>
</tbody>
</table>

# figures in parenthesis = % change from basal value, * P< 0.05 on Comparison with baseline record.
Fig. No. 10: 24 HOURS AMBULATORY BLOOD PRESSURE MONITORING: CHANGES IN SYSTOLIC AND DIASTOLIC BLOOD PRESSURE ON TREATMENT WITH COMBINATION OF LOSARTAN AND AMLODIPINE
Table No. 3B: CHANGES ON TREATMENT WITH COMBINATION OF LOSARTAN AND AMLODIPINE DAY TIME RECORDINGS

<table>
<thead>
<tr>
<th>% of reading above period limits</th>
<th>% of time the reading were above period limits</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Systolic</strong></td>
<td><strong>Diastolic</strong></td>
</tr>
<tr>
<td>B</td>
<td>A+L</td>
</tr>
<tr>
<td>3.5</td>
<td>1.1</td>
</tr>
<tr>
<td>Change</td>
<td>-2.4 (68)</td>
</tr>
<tr>
<td><strong>Diastolic</strong></td>
<td><strong>A+L</strong></td>
</tr>
<tr>
<td>6.4</td>
<td>0.81</td>
</tr>
<tr>
<td>Change</td>
<td>-5.6 (87.2)</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>% of reading above period limits</th>
<th>% of time the reading were above period limits</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Systolic</strong></td>
<td><strong>Diastolic</strong></td>
</tr>
<tr>
<td>B</td>
<td>A+L</td>
</tr>
<tr>
<td>2.6</td>
<td>4.8</td>
</tr>
<tr>
<td>Change</td>
<td>2.2 (83.8)</td>
</tr>
<tr>
<td><strong>Diastolic</strong></td>
<td><strong>A+L</strong></td>
</tr>
<tr>
<td>5.7</td>
<td>3.3</td>
</tr>
<tr>
<td>Change</td>
<td>-2.4 (42)</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Systolic Blood Pressure</th>
<th>Diastolic Blood Pressure</th>
<th>Mean arterial pressure</th>
<th>Heart rate</th>
</tr>
</thead>
<tbody>
<tr>
<td>B</td>
<td>A+L</td>
<td>B</td>
<td>A+L</td>
</tr>
<tr>
<td>118</td>
<td>111.9*</td>
<td>73.6</td>
<td>67.5*</td>
</tr>
<tr>
<td>Change #</td>
<td>-6.1 (5.2)</td>
<td>-6.1 (8.2)</td>
<td>-3.4 (3.8)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>-1.3 (1.7)</td>
</tr>
</tbody>
</table>

# figures in parenthesis = % change from basal value P<0.05 on Comparison with baseline record.
Fig. No. 11: 24 HOUR AMBULATORY BLOOD PRESSURE MONITORING: DAY TIME CHANGES IN SYSTOLIC AND DIASTOLIC BLOOD PRESSURE ON TREATMENT WITH COMBINATION OF LOSARTAN AND AMLODIPINE.
## AMBULATORY BLOOD PRESSURE

**Table No. 4B: CHANGES ON TREATMENT WITH COMBINATION OF LOSARTAN AND AMLODIPINE: NIGHT TIME RECORDINGS**

<table>
<thead>
<tr>
<th>% of reading above period limits</th>
<th>% of time the reading were above period limits</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Systolic</td>
</tr>
<tr>
<td></td>
<td>Systolic</td>
</tr>
<tr>
<td></td>
<td>B</td>
</tr>
<tr>
<td></td>
<td>B</td>
</tr>
<tr>
<td></td>
<td>7.9</td>
</tr>
<tr>
<td></td>
<td>4.6</td>
</tr>
<tr>
<td><strong>Change #</strong></td>
<td><strong>+3.2 (40.2)</strong></td>
</tr>
<tr>
<td><strong>Systolic Blood Pressure</strong></td>
<td><strong>Mean arterial pressure</strong></td>
</tr>
<tr>
<td></td>
<td><strong>B</strong></td>
</tr>
<tr>
<td></td>
<td><strong>B</strong></td>
</tr>
<tr>
<td></td>
<td><strong>104.7</strong></td>
</tr>
<tr>
<td></td>
<td><strong>63.3</strong></td>
</tr>
<tr>
<td><strong>Change #</strong></td>
<td><strong>-0.9 (0.8)</strong></td>
</tr>
</tbody>
</table>

* figures in parenthesis = % change from basal value
* P < 0.05 on comparison with baseline record N = 12
Fig. No. 12: 24 HOURS AMBULATORY BLOOD PRESSURE MONITORING: NIGHT TIME CHANGES IN SYSTOLIC AND DIASTOLIC BLOOD PRESSURE ON TREATMENT WITH COMBINATION OF LOSARTAN AND AMLODIPINE.

- Treatment Groups: B, A+L
- SBP: 100, 99.2
- DBP: 88
<table>
<thead>
<tr>
<th>Group</th>
<th>B</th>
<th>L</th>
<th>L+A</th>
</tr>
</thead>
<tbody>
<tr>
<td>N =</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>VR 79.2</td>
<td>77.3</td>
<td>76.9</td>
<td></td>
</tr>
<tr>
<td>± SD 7.5</td>
<td>6.8</td>
<td>8.3</td>
<td></td>
</tr>
<tr>
<td>Change # -1.9 (2.3)</td>
<td>-2.3 (2.9)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>PR Interval 111</td>
<td>129</td>
<td>125</td>
<td></td>
</tr>
<tr>
<td>± SD 9.4</td>
<td>0.03</td>
<td>0.01</td>
<td></td>
</tr>
<tr>
<td>Change # -18 (16.2)</td>
<td>-14 (12.6)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>QRS Duration 77</td>
<td>68</td>
<td>79</td>
<td></td>
</tr>
<tr>
<td>± SD 0.01</td>
<td>0.01</td>
<td>0.01</td>
<td></td>
</tr>
<tr>
<td>Change # -9 (11.7)</td>
<td>± 2 (2.6)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>QTC Interval 386</td>
<td>416</td>
<td>390</td>
<td></td>
</tr>
<tr>
<td>± SD 0.06</td>
<td>0.05</td>
<td>0.05</td>
<td></td>
</tr>
<tr>
<td>Change # +30 (7.7)</td>
<td>+4 (1.0)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

PR interval, QRS duration, QTC in milliseconds and ventricular rate (VR) in beats/min) B = Baseline, ventricular rate calculated from mean PR interval # figures in parenthesis = % change from basal values P < 0.05 On comparison between L and L+A.
# SYSTOLIC TIME INTERVALS

**Table 6B: EFFECT OF LOSARTAN AND COMBINATION OF LOSARTAN AND AMLODIPINE ON ELECTROMECHANICAL SYSTOLE, LEFT VENTRICULAR EJECTION TIME AND PRE-EJECTION PERIOD**

<table>
<thead>
<tr>
<th>Group</th>
<th>B</th>
<th>L</th>
<th>L+A</th>
</tr>
</thead>
<tbody>
<tr>
<td>N =</td>
<td>12</td>
<td></td>
<td></td>
</tr>
<tr>
<td>QS₂ index</td>
<td>570.7</td>
<td>546.3</td>
<td>538.3</td>
</tr>
<tr>
<td>± SD</td>
<td>20.3</td>
<td>16.2</td>
<td>17.1</td>
</tr>
<tr>
<td>Change #</td>
<td>-24.4 (4.3)</td>
<td>-32.4 (5.7)</td>
<td></td>
</tr>
<tr>
<td>LVET Index</td>
<td>415.2</td>
<td>405.8</td>
<td>409.8</td>
</tr>
<tr>
<td>± SD</td>
<td>15.4</td>
<td>18.2</td>
<td>20.4</td>
</tr>
<tr>
<td>Change #</td>
<td>-9.4 (2.2)</td>
<td>-5.4 (1.3)</td>
<td></td>
</tr>
<tr>
<td>PEP Index</td>
<td>141.8</td>
<td>124.9</td>
<td>128.5</td>
</tr>
<tr>
<td>± SD</td>
<td>14.5</td>
<td>20.2</td>
<td>21.4</td>
</tr>
<tr>
<td>Change #</td>
<td>-16.9 (11.9)</td>
<td>-13.3 (9.4)</td>
<td></td>
</tr>
<tr>
<td>LVET/PEP</td>
<td>0.33</td>
<td>0.34</td>
<td>0.33</td>
</tr>
<tr>
<td>± SD</td>
<td>0.05</td>
<td>0.07</td>
<td>0.07</td>
</tr>
<tr>
<td>Change #, $</td>
<td>0.01 (3.0)</td>
<td>0 (0)</td>
<td></td>
</tr>
</tbody>
</table>

QS₂ = Electromechanical systole, LVET = LEFT Ventricular ejection time PEP = Pre - Ejection Period
LVET/PEP = LVET:PEP Ratio, B = Baseline # figures in parenthesis = % change from basal value $% Change was always calculated before rounding off * p < 0.05, **P < 0.01 ***P < 0.001 on comparison of losartan (L) with L+A.
Fig. No. 14: PERCENTAGE CHANGES IN SYSTOLIC TIME INTERVALS ON TREATMENT WITH LOSARTAN (L) AND AMLODIPINE(A) AS COMPARED TO THE BASE LINE
SAFETY EVALUATION

1. Body weight record

The weight of the subjects/patient was recorded at every visit on a validated electronic weighing machine. In the amlodipine with losartan add on arm there was no change in the mean weight of the subjects. When losartan was administered first followed by amlodipine add on a small decrease of 0.3% was noted. None of the observations achieved statistically significance (Table no.1C).

There was no change in the body weight of the one hypertensive subject throughout the study period.

2. Adverse Events

Both spontaneously reported adverse events and those elicited by active questioning were recorded. A total of 5 adverse events were reported by the subjects in the amlodipine arm while those in the losartan arm reported 9 adverse events.

In the amlodipine followed by losartan add on there were three complaints of headache and two complaints of easy fatiguability.

Three instances of thoracic pain, two of cough with sputum, two each of easy fatiguability and headache were reported in subject taking losartan with amlodipine add on.

No complaint except thoracic pain required intervention. Liver function tests were done in all subjects subsequently but they were found to be within normal limits in all the volunteers including the three volunteers who had complained of thoracic pain. The
medication was not discontinued in these 3 volunteers and the pain subsided on its own.

Two volunteers one each in the amlodipine and losartan arm suffered from common cold and were prescribed steam inhalation and nasal decongestant for 3 days.

The hypertensive patient reported headache on two occasions and was prescribed analgesic medication.

Laboratory parameters

Table No. 2C, lists the various laboratory parameters estimated during the course of the study. These estimations took place at the end of the treatment with placebo, single active and two active drugs. There was no clinically significant effect observed in any of the treatment arms.

In the hypertensive patient no significant change was seen in any of the laboratory parameters.
WEIGHT RECORD

Table No. 1C: CHANGE IN WEIGHT ON TREATMENT WITH AMLODIPINE AND LOSARTAN, LOSARTAN AND AMLODIPINE

<table>
<thead>
<tr>
<th>Group</th>
<th>B</th>
<th>A+L</th>
<th>B</th>
<th>L+A</th>
</tr>
</thead>
<tbody>
<tr>
<td>N =</td>
<td>12</td>
<td>12</td>
<td>11</td>
<td>11</td>
</tr>
<tr>
<td>Weight</td>
<td>66.7</td>
<td>66.7</td>
<td>58.6</td>
<td>58.4</td>
</tr>
<tr>
<td>± SD</td>
<td>4.8</td>
<td></td>
<td>3.6</td>
<td></td>
</tr>
<tr>
<td>Change #</td>
<td>0 (0)</td>
<td>0.2 (0.3)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Weight in Kilograms, # in Parentheses = % Change from Basal Value.
Table No. 2C: CHANGES ON TREATMENT WITH AMLODIPINE AND COMBINATION OF AMLODIPINE AND LOSARTAN (n = 12)

<table>
<thead>
<tr>
<th>Group</th>
<th>B</th>
<th>A</th>
<th>A+L</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hemoglobin gm/dl (14-18)</td>
<td>15.9</td>
<td>16.1</td>
<td>16.3</td>
</tr>
<tr>
<td>Haematocrit % (33.1-35.7)</td>
<td>33.6</td>
<td>34.2</td>
<td>34.5</td>
</tr>
<tr>
<td>S. Sodium mmoles/l (136-145)</td>
<td>143</td>
<td>144</td>
<td>144.2</td>
</tr>
<tr>
<td>S. Potassium mmoles/l (3.5-5.1)</td>
<td>4.2</td>
<td>4.0</td>
<td>3.6</td>
</tr>
<tr>
<td>S. uric acid mg/dl (3.5-7.0)</td>
<td>4.0</td>
<td>4.2</td>
<td>4.4</td>
</tr>
<tr>
<td>Total Bilirubin mg/dl (&lt;1.0)</td>
<td>0.76</td>
<td>0.81</td>
<td>0.85</td>
</tr>
<tr>
<td>AST IU/L (15-37)</td>
<td>20</td>
<td>20.1</td>
<td>21</td>
</tr>
<tr>
<td>ALT IU/L (30-65)</td>
<td>32</td>
<td>31</td>
<td>36</td>
</tr>
<tr>
<td>ALPIU/L (50-136)</td>
<td>105</td>
<td>116</td>
<td>103</td>
</tr>
</tbody>
</table>

Figures in parenthesis depict the normal lab range for that parameter.
# LABORATORY PARAMETERS

Table No. 2D: CHANGES ON TREATMENT WITH LOSARTAN AND COMBINATION OF AMLODIPINE AND LOSARTAN (N = 11)

<table>
<thead>
<tr>
<th>Group</th>
<th>B</th>
<th>L</th>
<th>A+A</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hemoglobin gm/dl (14-18)</td>
<td>16.2</td>
<td>15.6</td>
<td>15.4</td>
</tr>
<tr>
<td>Haematocrit % (33.1-35.7)</td>
<td>34.2</td>
<td>34.7</td>
<td>34.9</td>
</tr>
<tr>
<td>S. Sodium mmoles/l (136-145)</td>
<td>138</td>
<td>142</td>
<td>144</td>
</tr>
<tr>
<td>S. Potassium mmoles/l (3.5-5.1)</td>
<td>4.6</td>
<td>3.8</td>
<td>3.7</td>
</tr>
<tr>
<td>S. uric acid mg/dl (3.5-7.0)</td>
<td>4.2</td>
<td>5.0</td>
<td>4.6</td>
</tr>
<tr>
<td>Total Bilirubin mg/dl (&lt; 1.0)</td>
<td>0.39</td>
<td>0.55</td>
<td>0.62</td>
</tr>
<tr>
<td>AST IU/L (15-37)</td>
<td>22</td>
<td>21</td>
<td>24</td>
</tr>
<tr>
<td>ALT IU/L (30-65)</td>
<td>32</td>
<td>36</td>
<td>35</td>
</tr>
<tr>
<td>ALPIU/L (50-136)</td>
<td>103</td>
<td>105</td>
<td>110</td>
</tr>
</tbody>
</table>

Figures in parenthesis depict the normal lab range for that parameter.
BETWEEN GROUP COMPARISONS

The changes resulting upon administration of amlodipine and losartan and losartan and amlodipine did not differ in a statistically significant manner.

Patient Data: The data of the hypertensive patient is not shown, since the patient was non-compliant and it would not have been possible to draw valid conclusions. The data however is available in the form of raw data.
DISCUSSION
Coronary heart disease represents a substantial burden of global mortality and morbidity. Current estimates suggest that, by the year 2020, coronary heart disease will be the leading cause of death worldwide and cerebrovascular disease will be the fourth most common cause. (Caroia Bardage, 2000)

Hypertension is the most important contributing factor to this rise in morbidity and mortality. It has been shown that antihypertensive treatment, resulting in significant lowering of diastolic blood pressure, reduces the occurrence of fatal and non-fatal stroke and coronary heart disease; (Collins et al., 1990). The value of effectively treating hypertension is thus clearly established.

The current trend in the treatment of mild and moderate hypertension has been the use of monotherapy but it has been seen that the usual reductions in blood pressure by monotherapy would not restore optimal or even non-hypertensive blood pressure levels in many patients (WHO-ISH Guidelines 1999). On the other hand combination therapy of several of the available drug classes has been shown to produce blood pressure reductions that are greater than those produced by any group of individual agents used alone (Menard, 1993).

A number of intervention trials in hypertension have shown that patients requiring combination therapy varies from 33% to 100% (Mancia et al., 1997). Another example is the recently concluded HOT study, in which diastolic blood pressure was lowered to below 90mm
Hg in over 90% of patients, demonstrated that combination therapy was necessary in 70% of participants. (Hansson et al., 1998).

Combination therapy has also been recommended for the management of refractory hypertension, where blood pressure remains uncontrolled despite sustained therapy with at least three different classes of anti hypertensive agents. (Alper et al., 1999)

Recent guidelines JNC VI (1997) and WHO-ISH guidelines (1999) recommend the use of combination therapy over monotherapy as it enhances compliance through cost savings, more convenient dosing and reduced pill burdens and since it allows for use of both the drugs in low doses, the incidence of adverse effects is also reduced.

Amidst the various combinations that are currently available, the combination of a calcium channel blocker and an ACE inhibitor or Angiotensin II receptor antagonists seems to be very promising and is being advocated in an increasing number of clinical situations. (Roberto 1997; Messerli et al., 2000; Kloner et al., 2001).

With this background a literature search relating to the combined use of these two classes of drugs was done. A computer search on PUBMED: National Library of Medicine, did not reveal any long-term study conducted in normotensive subjects probing the hemodynamic consequences of a combination of calcium channel blocker and Angiotensin II receptor antagonist even when a fixed dose combination of amlodipine (CCB) and losartan (Ang II AT1 antagonist) is available in the market today. (The computer search was conducted till August 2001, with key words, angiotensin converting
enzyme, calcium channel blocker, amlodipine, losartan. Combination, hypertension, normal healthy volunteers).

It was therefore, decided to evaluate the hemodynamic consequences of the administration of a combination of calcium channel blocker-amlodipine and Angiotensin II receptor antagonist -losartan in normal healthy volunteers and if feasible the antihypertensive efficacy of the same combination in patients with mild to moderate hypertension.

The conduct of the study has a number of aspects that merit discussion. Most of the principles envisaged in the Good Clinical Research Practice (ICH Guidelines, 1997) were adhered to. The study protocol was approved by Jamia Hamdard Institutional Review Board. Each of the subjects/patient was required to read understand and affix his signature on the written informed consent form. The signed original ICF was retained and a copy of the same was given to each participant. The standard SOPs of the Ranbaxy CPU have been adhered to while recording all the parameters. Two placebo formulations(double- dummy) were employed in the conduct of the study. This was necessary in order to match the different study drugs. A 2.5 mg placebo tablet substituted for amlodipine and 50 mg tablet for losartan.

The subjects /patient began their placebo run-in period receiving a placebo formulation that was to be replaced by an active medication in the last two weeks of the trial. After the first week, the subject received two drugs (one active and one placebo) for two weeks. For the last two weeks of the trial placebo was replaced by the second
active medication. As the subjects were receiving a placebo mimicking this second active medication, its replacement would prevent any response just because the act of addition of another drug. It is, therefore, expected that placebo response has been adequately taken care off throughout the study.

Randomization schedule was computer generated by SAS software. The randomization schedule was kept with the supervisor who deputed a colleague. Drug dispensation and pill counting was always done by this colleague and the observer was never aware as to what medication a given subject was receiving. The subjects too were unaware as to which particular medications they were receiving. Thus double blinding was ensured. The study in the hypertensive patient was conducted in a single blind manner and the patient was unaware as to which particular medication she was receiving. All the subject/patient were asked to take the medications in the morning between 0800-0900 hours. Except for seven instances all the other medication events were reported to have occurred within this time period. In the seven other instances the last time point for medication was 1030 hours.

All the subjects/patient were highly cooperative throughout the conduct of the study and a very high compliance to medication was obtained. All the subjects/patient met the requisite compliance criteria. Two subjects did not take medication on one day each while the hypertensive patient did not take medication on two days.
A single observer recorded all the observations. If the observation required the participation of more than one person, as in the conduct of the systolic time intervals, the observer was the same for a subject throughout the study period.

All the subjects/patient were asked to report to the CPU at 0800 hours for the hemodynamic evaluation days and for the application of the ambulatory blood pressure monitor at 0900 hrs. Thus all the observations were recorded at almost the same time of the day. Moreover, during each visit care was taken to ascertain that there was no major change in the routine of the subject evaluation. As the randomization schedule was generated inclusive of two arms of this parallel designed study; the two combinations have been evaluated simultaneously.

The design also addressed the possible order effect of drug administration. In each arms of the treatment, half the number of participants were randomised to receive a calcium channel blocker (amlodipine) initially and the other half received an Angiotensin II AT1 receptor antagonist (losartan) first. The hypertensive patient received amlodipine first followed by losartan. The subjects thus received two weeks of treatment with the single anti-hypertensive drug and a placebo. And the placebo was then replaced with the other anti-hypertensive drug such that each subject/patient received a combination of amlodipine and losartan for the remaining two weeks of the trial. The main objective of such a design was to overcome any effect due to the order of addition of the anti-hypertensive drugs.
BLOOD PRESSURE LOWERING EFFECT

The results indicate that on an average losartan has caused a minor fall in blood pressure. There was a distinctly greater fall, in the diastolic blood pressure as compared to systolic blood pressure, in both supine and standing postures. The fall in systolic blood pressure was almost identical in both the supine and standing postures (1.1% and 1.3%) Diastolic blood pressure, however was reduced to a greater extent in the standing position than in the supine posture (3.6% and 1.7% respectively).

This reduction of blood pressure in both these postures was accompanied by an increase in heart rate of +5.7% in supine posture and +4.8% in standing posture, both of which were statistically not significant.

There are reports of reduction of blood pressure by the administration of losartan to healthy volunteers. In one study (Goldberg et al., 1995), effects of losartan on blood pressure, plasma renin activity and Angiotensin II were studied in volunteers. 100 mg losartan was administered for a week and the fall in supine systolic and diastolic blood pressure observed was 11.6 ± 8 and 7.0 ± 4.8 mn Hg respectively after the last dose.

However in several other later studies, in healthy volunteers losartan was well tolerated but no detectable falls in blood pressure were identified. Even with optimal designs and double blind cross over trials, there appears to be very little effect of losartan upto 120 mg on
blood pressure of normal subjects after single doses and upto 10 days daily administration. (McIntyre et al., 1997). As the renin-angiotensin system in normal subjects is not activated, these findings are not surprising.

The fall in blood pressure is much greater in normal volunteers in whom the renin-angiotensin system is activated either by angiotensin II infusion or by a low sodium diet and furosemide administration before the study.

The inhibitory effect of losartan has been studied on the pressor action of exogenous angiotensin I and II in healthy male volunteers (Christen et al., 1991). Systolic blood pressure response to test doses of angiotensin II was reduced to 37 ± 7%, 40 ± 4% and 38 ± 6% of baseline values on days 1, 4 and 8 respectively.

In another study by Doig et al (1993) losartan was administered orally to normal volunteers whose renin-angiotension system had been activated by a low sodium diet (40 mmol) and furosemide (40 mg twice daily) for 3 days before the study. Statistically significant decreases in supine and erect posture blood pressures were observed with 50 mg and 100 mg dose administered for 3 days.

As expected there was no significant effect of losartan on the heart rate. A small increase was however noted in both the postures. The reason attributed for the lack of reflex tachycardia is an increase in either central or peripheral parasympathetic activity, without the
impairment of baro-receptor or sympathetic function (Ajayi et al., 1986).

Amlodipine in the present study caused a very small reduction of 1.6% and 0.69% in the supine systolic and diastolic blood pressures respectively. In erect posture the fall in systolic blood pressure was 0.8% and in diastolic blood pressure it was 1.4%. There was a small increase in the supine and standing heart rate by +3.7% and +3.6% respectively.

This finding is corroborated by recent study (Lay et al., 2001) where the effect of 10 mg daily dose of amlodipine for two weeks, was studied on the cardiopulmonary performance in volunteers. There was no change in the blood pressure levels or heart rate in these volunteers.

One earlier report of amlodipine indicates that single dose administration of 5 mg had no effect on resting heart rate and mean arterial pressure (Goldsmith, 1995).

On the addition of a second drug, a complimentary further reduction in the blood pressure was observed in both the groups. The total reduction in the blood pressure achieved considering the groups as a whole was more than the sum of the fall caused by the individual drugs i.e., a synergistic interaction was observed.

In the amlodipine arm, the fall achieved by combined administration was 2.2% in supine systolic and 2.3% in supine diastolic blood
pressures. The rise in supine and standing heart rates was +4.8% and +5.2% respectively.

In the losartan arm, the fall achieved by amlodipine add on was 1.7% in supine systolic blood pressures and 5.7% in supine diastolic blood pressures, while in standing systolic and diastolic the fall was 3.1% and 6.4% respectively. The fall in supine diastolic blood pressures was more in the group which received losartan initially followed by amlodipine add on compared to the other group which received amlodipine initially followed by losartan add on. An order effect was thus observed in the combined anti-hypertensive action. This can be easily explained by the fact that amlodipine per se caused hardly noticeable fall in supine diastolic blood pressure (0.69%). It thus appears that losartan has a greater effect on the supine diastolic blood pressure in healthy volunteers.

The fall in standing diastolic blood pressure is more in the second group (losartan with amlodipine add on) 6.4% but since the fall was less than 10mm Hg it cannot be termed as orthostatic hypotensive effect.

Although orthostatic effects and first dose hypotension have been reported in losartan recipients, the incidence is very low ≤ 0.5% after 25 or 50mg dosages. (Simpson et al., 2000).

Ambulatory Blood Pressure Monitoring

This was done once at the end of placebo run-in period and then at end of combination therapy. Thus there is no data available for
comparison of the effects of individual drugs. Ambulatory data monitoring provides a picture of the blood pressure control over the entire day as the subject continues to engage in the normal routine activities. Care was also taken to acclimatize the subjects/patient with blood pressure monitoring by applying the instrument to the subjects/patient during their first visit before the initiation of the placebo therapy. This was done to avoid the possible initial anxiety and discomfort interfering with the blood pressure recordings. The blood pressure control exhibited typical circadian changes. The blood pressure is minimum at midnight, starts rising at about 0400 hrs and reaches a maximum between 0800-0100 hrs (Elliot, 1996). Such a typical circadian variability was evident in the ABP recording obtained in the present study.

There is some correlation between the clinic blood pressure data and Ambulatory blood pressure data.

<table>
<thead>
<tr>
<th>Drug combinations</th>
<th>Ambulatory BP (% change)</th>
<th>Clinic blood pressure (% change)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>SBP</td>
<td>DBP</td>
</tr>
<tr>
<td>A+L</td>
<td>-4.3</td>
<td>-2.9</td>
</tr>
<tr>
<td>L+A</td>
<td>-3.9</td>
<td>-7.9</td>
</tr>
</tbody>
</table>

In the amlodipine arm the fall in systolic blood pressure is more than in the losartan arm as seen both by clinic blood pressure and ambulatory blood pressure monitoring.
On the other hand the fall in diastolic blood pressure is more in losartan arm as compared to the amlodipine arm both in terms of clinic blood pressure measurement and diastolic load (-5.7% and -7.9% respectively). Thus we can conclude that a definite order effect is seen in the combined antihypertensive action of amlodipine and losartan.

The combination exhibited efficacy both during the day time as well as during the night time as is evident from ambulatory blood pressure monitoring data.

This is easily explainable as amlodipine is a long acting dihydropyridine antagonist with a half-life of 36 hours to 50 hrs and losartan though has a short half-life of 2.1 hr but its active metabolite E 3174 has a half-life of 6.3 hr.

A study was performed to assess the residual antihypertensive effect of therapy in well-controlled mild to moderate hypertensive patients using conventional and ambulatory blood pressure monitoring (ABPM). It was found that the duration of action of amlodipine extends largely beyond the 24-h span, and when patients omit their treatment for 3 days BP does not significantly increase (Bistin et al., 1999).

Since it is known that one out of six non-compliant patient is on a drug holiday (>3 days of noncompliance), long acting drug or drug combinations like that of amlodipine and losartan possess distinct
advantages over short acting drugs when it comes to the potential harmful effect of "drug holidays".

**Electrocardiographic changes**

In the amlodipine arm there was no significant impact on any of the ECG parameters.

There was a minor decrease in the P-R interval when losartan was added to amlodipine but it was statistically insignificant.

Amlodipine per se produced a small decrease in QRS duration, while addition of losartan produced a small increase. Both these changes were statistically not significant. Similarly in the losartan arm, losartan per se produced an increase in the QTc interval by 11.7% while amlodipine per se produced a decrease by 3.7%, but this change is of little clinical relevance as the QTc interval is still within the normal range (Normal range 350-430 milliseconds). Addition of amlodipine to losartan produced an increase of only 2.6%, thus amlodipine decreased losartan induced increase in QTc interval. There was no significant change in any other E.C.G. parameter in the losartan arm.

**Systolic time intervals**

STI provides precise insight into the changes in cardiac performance in normal volunteers. Positive ionotropic agents like digoxin and digitoxin cause a shortening of all the STI indices – QS₂, LVET, PEP, PEP/LVET ratio. But positive ionotropism caused by an increase in
the calcium concentration shortens PEP and unlike digoxin lengthens LVET and QS \(_2\) indices. Negative ionotropic agents like quinidine increase all these indices. Drugs like nifedipine despite a reduction in the afterload, cause a lengthening of QS \(_2\) index lengthening of LVET and shortening of PEP reflecting their negative ionotropism (Richard et al., 1977; Belz, 1993).

Somewhat similar effect is seen in our study in the amlodipine arm. There was an increase in QS \(_2\) by 2.5% on administration of amlodipine. A very small increase in LVET index (0.59%) and a decrease of 10.5% in the PEP index. However there was no significant change in LVET/PEP ratio in the amlodipine arm.

In the losartan arm, there was a decrease in QS \(_2\) index by 4.3% on losartan administration. This change was associated with a decrease in LVET index and PEP index.

It has been reported that an increase in after-load by administration of angiotensin leads to an increase of QS \(_2\) index and PEP index and shortening of LVET. (Belz, 1993). Losartan by blocking the action of angiotensin results in a decrease in QS \(_2\) index, which is observed in our study, although there is no accompanied decrease in PEP index and increase in LVET index.

In the present study, in the lone hypertensive patient, data was not enough to draw any valid conclusions. No synergism was seen in the clinical B.P. phase but additive effect was seen in the ABPM phase.
This discrepancy was due to non-compliance for 2 days in the clinical phase.

No significant changes were observed in ECG parameters and systolic time intervals. This stresses the need for ensuring compliance in patients on antihypertensives.

Safety Parameters

Both these combinations were well tolerated by the volunteers and the hypertensive patient. Three subjects on losartan monotherapy developed thoracic pain. Reports indicate (Nygaard et al., 1996) that losartan does cause a rise in liver enzymes leading to thoracic pain. But surprisingly, the liver function tests were within normal limits in all these three subjects.

Two subjects on amlodipine monotherapy complained of asthenia and generalized fatigue which is commonly associated with the use of amlodipine (Gonzalo et al., 1994).

The hypertensive patient reported headache at two instances, not a rare finding in patients receiving amlodipine (Gonzalo et al, 1994).

Only one hypertensive patient could be enrolled in this study. It is well known that adequately controlled hypertensive patients are reluctant to participate when informed about the placebo run-in phase of the clinical trial. Only one out of ten hypertensive patients screened gave informed consent to participate in this study.
Mechanism of Action

The complimentary reduction in blood pressure by amlodipine and losartan suggests a synergistic action.

Amlodipine is a dihydropyridine calcium antagonist which inhibits the transmembrane influx of Ca$_2^+$ through L channels in cardiac myocytes and smooth muscle cells of arteries. Because it is more selective for vascular smooth muscle than for cardiac muscle cells, amlodipine reduces peripheral vascular resistance without affecting cardiac conduction or myocardial contractility (Gonzalo et al., 1994).

Losartan on the other hand is an angiotensin II AT$_1$ receptor blocker. It binds to the AT$_1$ receptor thus inhibiting the action of angiotensin II, which is a powerful vasoconstrictor.

Renoprotective effect of calcium antagonists is well known and one of the reasons cited behind this action is that they are known to block angiotensin II mediated vasoconstriction in humans (Andrawis et al., 1992; Donati et al., 1992), thus complimenting the action of angiotensin II receptor antagonists.

Vasodilators are known to trigger renal compensatory mechanisms which lead to sodium and water retention, losartan has a marked natriuretic effect and can, therefore, take care of this response initiated by CCB.

The combination of amlodipine and losartan is beneficial in many more ways, like both these drugs are metabolically neutral and have
no significant effect on serum glucose or lipid levels. Not only this both are long acting drugs, amlodipine with a half life of 36-50 hrs and losartan has an active metabolite which has a half-life of 6.3 hrs. Therefore, both these drugs have a trough to peak ratio of > 50% much is very which desirable in the current context (Leszek et al., 1995). Complimentary mechanisms of action and prolonged duration of action makes this combination rational and synergistic.

LIMITATIONS OF THE PRESENT STUDY

The major limitation of the study is that the study has been conducted mainly in normotensive volunteers and only one hypertensive patient could be enrolled. Healthy salt-replete volunteers with intact homeostatic compensatory mechanism will show a response which will be different from the patients in whom these mechanism are impaired. Certainly if salt depleted volunteers (by low salt diet /furosemide) were used, more accurate assessment of hemodynamic efficiency of losartan could have been made. So ideally a cross over study in mild to moderately hypertensive patients will give an idea about the true efficacy of this combination.

Another limitation is the design of the study, amlodipine is a long acting drug with a half life of 36-50 hrs and it takes 4-6 weeks for its effect to stabilise, while in our study it has only been administered for two weeks, which may have been too short a time for amlodipine to show its complete effect. So administration of amlodipine for atleast 4 weeks is recommended for future studies.
Since the objective of the study was to compare monotherapy versus combination therapy in the management of hypertension, a control arm where subjects receive only monotherapy with the two study drugs would have been desirable. This type of a study design would have offered a better comparison between the two modalities. Limitation of the time available for completion of this thesis project was the reason for not selecting this study design.

The method used for assessing compliance has its inherent limitations. This being an outpatient study, one can never be sure whether the volunteer is taking the medication or not as one has to solely rely on the information he gives to the investigator. In these situations even pill count is an unsatisfactory method. Sophisticated methods like use of bottles with electronically controlled caps, that keep a record of the number of times the bottle is opened over a specified time period, would be much more accurate.