Available online at www.scholarsresearchlibrary.com

Scholars Research Library

Der Pharmacia Lettre, 2012, 4 (3):1027-1037
(http://scholarsresearchlibrary.com/archive.html)

Scholars Research Library
ISSN 0975-5071
USA CODEN: DPLEB4

Evaluation of toxicological and adverse effects produced by losartan

P. Polireddy*, S. Babuji, J. Venkateswararao, B. SridharReddy, S K. Shabber

---

ABSTRACT

Losartan is a specific angiotensin II receptor antagonist. Although the teratogenic effects of angiotensin converting enzyme (ACE) inhibitors are well documented there are limited reports of losartan induced fetal toxicity. The authors report a case of incomplete ossification of skull bones, transient oliguria and feed intolerance in a newborn following in-utero exposure to losartan. Losartan, an angiotensin II receptor antagonist, is widely used for the treatment of hypertension. Clinical experience with this drug has demonstrated that it is safe Losartan-induced hepatic toxicity is extremely rare. We report a case of severe hepatic toxicity and fibrosis caused by losartan use, and we review four previously reported cases. Drug-induced hepatic injury may be seen during the treatment of hypertension by losartan and the clinician should be aware of this toxicity, especially during the initial phase of treatment. The author has made a humble effort by this project work to bring it to the notice of the clinical people about the various adverse reaction organ toxicities produced by Losartan and thereby promoting pharmaco vigilance in clinical scenerio.

Keywords: Losartan, Thyrotoxicosis, Phaeochromocytoma, Primary' hypertension

---

INTRODUCTION

An adverse reaction has been defined by the world health organization as any response to a drug which is noxious, unintended and occurs at doses used in man for prophylaxis, diagnosis or therapy. Adverse drug reactions are an important cause of morbidity and mortality.[2] They are responsible for a considerable number of hospital admissions and significantly increase health costs. Important predisposing factors to adverse drug reactions include extremes of age, polypharmacy, intercurrent disease and genetic factors[3]. Mechanisms of reactions may be pharmaceutical, pharmacokinetic or pharmacodynamic.All drugs are capable of producing adverse effects and whenever a drug is given risk is taken. The magnitude of risk has to be considered along with magnitude of expected therapeutic benefit in deciding whether to use (or) not to use a particular drug for a given patient.

Predictable (Type A) reactions

These are qualitatively normal but augmented responses to drugs, such as bradycardia with a β adreno receptor blocker or hypoglycaemia with a sulphonyl urea.[4] Many type A reactions are due to a property of the drug which is unrelated to its primary therapeutic effect, such as gynaecomastia with cimetidine (or) dry mouth with phenothia zines.

These are usually predictable from the pharmacology of a drug. They are generally dose-dependent and although they are relatively dose-dependent common they don't generally cause serious illness.[5]

TOXICOLOGICAL STUDIES OF LOSARTAN

1. Losartan induced fetal toxicity

Losartan is a specific angiotensin II receptor antagonist. Although the teratogenic effects of angiotensin converting enzyme (ACE) inhibitors are well documented there are limited reports of Losartan induced fetal toxicity.[6] The authors report a case of incomplete ossification of skull bones, transient oliguria and feed intolerance in a newborn following in-utero exposure to Losartan .

2. Effect of experimental renal failure of the pharmacokinetics of Losartan in rats.

Aim:

The purpose of this investigation was to determine whether the pharmacokinetics of the angiotensin II receptor antagonist Losartan is altered in renal failure.

Male Wistar rats were pretreated with uranyl nitrate or subjected to bilateral ureteral ligation to produce acute renal failure(ARF). Saline-injected and sham-operated rats, respectively, served as controls. Uranyl nitrate-treated rats showed significantly higher serum concentrations of Losartan after oral administration and the area under the serum concentration-time curve (AUC(0-24)) of Losartan increased about 3-fold compared to control rats.

The systemic clearance of Losartan significantly decreased from 410+/- 254ml/h/kg in control to 177+/- 112ml/h/kg in uranyl nitrate-treated rats. In order to investigate the mechanisms of reduced clearance of Losartan associatd with ARF, a hepatic microsome fraction was prepared from normal and ARF rats. No significant difference was found in the metabolism of Losartan by hepatic microsomes prepared from ARF and control rats. In addition, the metabolic activity of microsomes was examined in the presence of uremic rat serum. The unbound clearance of Losartan and the unbound clearance associated with the formation of EXP3174 in the presence of uremic serum were significantly lower than those in the presence of control serum. Furthermore, the metabolism of Losartan was inhibited by indoxyl sulfate, a uremic toxin, in an uncompetitive manner.

3. Losartan -induced hepatic injury

Losartan , an angiotensin II receptor antagonist, is widely used for the treatment of hypertension. Clinical experience with this drug has demonstrated that it is safe Losartan -induced hepatic toxicity is extremely rare. We report a case of severe hepatic toxicity and fibrosis caused by Losartan use, and we review four previously reported cases. Drug-induced hepatic injury may be seen during the treatment of hypertension by Losartan and the clinician should be aware of this toxicity, especially during the initial phase of treatment.

ANGIOTENSIN ANTAGONISTS

Losartan

On June 1998 Losartan was introduced into the market in India for the treatment of hypertension. Losartan is described chemically as 2-butyl-4-chloro-1-[[2'-(1H-tetrazol-5-y1) [1,1'-biphenyl]-4-y1]methyl]-1H-imidazole-methanol, monopotassium salt. It is freely soluble in water. The empirical formula of Losartan $C_{22}H_{23}ClN_6O\bullet K$ and molecular weight is 461.0 and the structure is as follows. It is a competitive antagonist of AII devoid of partial agonistic activity and 10,000 times more selective for AT_1 than AT_2 receptor; does not block any other receptor or ion channel. It blocks all overt actions of AII viz. vasoconstriction, central and peripheral sympathetic stimulation, release of aldosterone and ADr from adrenals, renal actions promoting salt and water reabsorption, central actions like thrist, vasopressin release and growth promoting actions on heart and blood vessels. No inhibition of ACE or potentiation of bradykinin has been noted.[7]

TOXICOLOGY

Acute Toxicity

The oral LD_{50} of Losartan potassium in male is 2248 mg/kg (6744 mg/m^2). Significant lethality was observed in rat and rats after oral administration of 1000 mg/kg (3000 mg/m^2) .

ANIMAL TOXICOLOGY

Carcinogenesis

Losartan potassium was not carcinogenic when administered at maximum tolerated dosage levels to rats and rat for 105 and 92 weeks, respectively. These maximum tolerated dosage levels provided respective margins of systemic exposure for Losartan and its pharmacologically active metabolite over that achieved in humans treated with 50 mg of Losartan of approximately 270 – and 150- fold rats 45 – and 27- fold in rat.

MATERIALS AND METHODS

1. Preparation of the drug solution
Losartan was found freely soluble in water. Hence 0.1% of drug solution was prepared (100 mg of drug in 100 ml of water).

2. Acute toxicity study in albino rats to moniter
(a) LD_{50} determination :-
Animal used :- Albino rat
Weight :- 150 –200gm.
Procedure
The acute toxic method is a step wise procedure with 3 animals of a single per step. Depending on the mortality and / or moribund status of animals on the average 2-4 steps necessary to allow the judgement on the acute toxicity of the test substance[8]. This procedure results in the use of minimal number of animals while allowing for acceptable data based scientific conclusion.

The method was defined dose (500, 1000, 2000, 3000, 4000, mg/kg body weight) and the results allows a substance to be ranked and classified according to the Globally Harmonized systems (GHS) for the classification of chemical which cause acute toxicity.

Thirty male wister rats weight 150-200gm was used for study. The starting dose level of Losartan was 4000 mg/kg body weight. As most of the Losartan posses LD_{50} vaule more than 2000 mg/kg. Dose volume was administered 0.5ml/10gm body weight to the rat which was fasted over night with water ad *libitum.* Food was withheld for a further 3-4 hours after administration of the drug.

2. (b) Gross behavioral studies
After IP administration of compounds to groups of 6 rat each the animals were observed for gross behavioural effects. The animals are observed continuous for 3 hours after administration of the compound then every 30 minutes for next 3 hrs and finally after 24 hours. CNS stimulation is judged by increased spontaneous motor activity (SMA), Piloerection, exopthalmous, clonic and (or) tonic convulsions, CNS depressions, Judged by reduced SMA, sedation, crouching, catalepsy and autonomic effects like piloerection, urination, defecation, salivation, lachrymation etc.

2 (c) Histopathological changes
At the end of the acute toxicity study, the animals were killed by stunning liver, Kidney, brain lungs and heart were identified and examined for macroscopic changes. These organs were preserved in 10 % formalin dehydrated with ascending grade of ethylacohol, embedded in paraffin wax, sliced on a rotary microtoma stained with haemotoxylin and eosin and histomorophological features were examined.

3. Sub-acute toxicity study in albino-rats to monitor
a. Bio-chemical changes
Sub – acute toxicity
Animal model
Twelve, random bred, male albino rats weighing 150 – 200 gms were caged and maintained on standard laboratory diet adlibitum.

Group I- control group (Total no of rats –6)
All the rats were fed vehicle (0.9% Nacl saline). For 15days in the calculated volume.

Group II- Drug treated group (Total no of rats –6)
a. Consists of 6 rats and were treated with Losartan (0.1mg/kg) (Dose equivalent to therapeutic dose of Losartan daily intraperitonelly for 5 days.
b. Consists of rats and were treated with Losartan (0.2mg/kg) (Dose equivalent to 2 times that of the therapeutic dose of Losartan) daily intraperitoneally for 6-10 days.
c. Consists of 6 rats and were treated with Losartan (0.4mg /kg) (Dose equivalent to 4 times that of the therapeutic dose of Losartan) daily intraperitoneally for 11-15 days.ll animals were given measured amount of food and water daily. Period of study was 15 days. During the study, body weight and food intake were measured. Blood was collected at the end of 15^{th} day and estimations of serum alkaline phosphatase, Bilirubin, SGOT, SGPT were determined and compared with those of control animals.[9]

3. (a) Bio-chemical Studies

Collection of blood for the various estimations

Blood was collected for individual rats by retro orbital bleeding.

I. ESTIMATION OF ALKALINE PHOSPHATE LEVEL

Principle

Serum ALP hydrolyzes disodium phenyl phosphate into phenol and disodium hydrogen phophate at pH 10.0. The phenol so formed reacts with 4-Aminoantipyrine in alkaline medium in presence of oxdizing agent Potassium ferricyanide to form a red coloured complex whose absorbance is proportional to the enzyme activity.

Procedure

1ml of working Buffered substrate add 3ml of Deionized water and incubate for 3 minutes at 37°C. And add 0.1ml of serum incubate for 15 minutes at 37°C. After that 2ml of coloured reagent mix well and measure the absorbance at 510nm.

II. ESTIMATION BILIRUBIN

Principle

Bilirubin reacts with diazotized sulfanilic acid in acidic medium to form azobilirubin, a purple colored complex whose absorbance is proportional to Bilirubin concentration. Direct Bilirubin, being water soluble is allowed to react with diazotized sulfanilic acid in the absence of an activator, while for total Bilirubin (Direct & Indirect) the diazotization is carried out in the presence of an activator.

Procedure

Take 2 clean dry test tuby labeled as T1, T2. And add tube reagents one by are i.e Diazo-A 1ml, Diazo-B 0.1ml Activator 1ml Distilled water 2.5 ml in T1, 2.6ml in T2 and serum 0.2ml mix well and read the absorbance at 540nm.[9]

III. ESTIMATION SGOT

Principle

SGOT catalyzes transfer of amino group from L-alainine to α- ketoglutarate with formation of oxaloacetate & glutamate. The oxaloacetate so formed, is allowed to react with 2.4 DNPH to produce 2,4 dinitro phenyl hydrazones derivative which is brown colored in alkaline medium. The absorbance of this hydrazone derivative is correlated to SGOT activity plotting a calibration curve using pyruvate standard.[10]

Procedure

0.5ml of Buffered substrate is incubate at 37°C for 3 minuts and add 0.1 ml of serum mix well and incubate at 37°C for 60 minutes. Then add 0.5ml of DNPH colour reagent and 5ml of working sodium hydroxide mix well and allow 10 minuts and measure the absorbance at 505 nm.

IV. ESTIMATION OF SGPT

Principle

SGPT catalyzes transfer of amino group from L- alanine to α- ketoglutarate with formation of pyruvate & glutamate. The pyruvate so formed, is allowed to react with 204 DNPH to produce 2,4 dinitrophenyl hydrazone derivative which is brown coloured in alkaline medium. The absorbance of this hydrazone derivative is correlated to SGPT activity by plotting a calibration curve using pyruvate standard.

Procedure

0.5ml of Buffered substrate is Incubate 37°C for 3 minuts and add 0.1ml of Serum and incubate at 37°C for 30 minuts and add 0.5ml of DNPH color Reagent, 5ml of Working soduium Hydroxide mix well and allow to stand at room temparature for 10 minuts and measure the absorbance on spectrophotometer at 505nm.

4. CARDIOVASCULAR EFFECTS OF LOSARTAN

4.1 Isolated frog Heart Preparation.

Experimental workdone

Isolated frog heart preparation.

Requirements

Animal : Frog

Weight : 150-200 gm

Physical solution: Frog Ringer solution

Procedure
Isolated frog heart preparation was putup using symes cannula. Losartan was added into the preparation in doses of 5µ, 10µ and their effects recorded. Losartan (40µ) was repeated against after the administration of propranolol (20µ) and the results discussed.

5.2 Effect on perfused blood vessels of frog
Requirements
Animal : Frog
Weight : 150-200gm
Physiological solution : Frog Ringer solution

Procedure:
Dissected the frog and ligated one branch of aorta and carried out cannulation in the second branch of the aorta and perfused frog ringer solution through it. Made a cut in the inferior venacava, and inserted the venous cannula in the opposite direction of the heart. Normal outflow of perfusate was recorded through the venous cannula. Adjusted the flow of perfusion to 20 drops per minute by a screw clip administered at different concentration through the rubber tube attached to the venous cannula. After administration of the drug the volume of perfusate coming out from inferior venacava after 0.5, 1.0, 1.5, 2.0 minutes for 3 minutes were measured.

The drug were given in the following manner

Adrenaline	10 µg / ml	Losartan	100 µg/ml
Acetylcholine	50 µg / ml	Losartan	300 µg/ml
Nitroglycerin	1%	LosarTan	400 µg/ml

RESULTS AND DISCUSSION

1. ACUTE TOXICITY STUDY IN ALBINO RAT TO MONITOR LD_{50} DETERMINATION

Animal Used : Albino rat
Weight : 150 – 250gm
Route of Administration : Intra peritoneal route
Drug : Losartan

TABULATE COLUMN -1

Groups	No.of animals	Body weight	Concen-tration dose (mg/kg)	Log dose	Dead /Total	% of death	% of correction	Probit value
I	6	200	500	2.7	0/6	0	4.17	3.27
II	6	210	1000	3	2/6	33.3	33.3	4.56
III	6	210	2000	3.30	3/6	50	50	5.00
IV	6	190	3000	3.48	5/6	83.3	83.3	5.41
V	6	200	40000	3.60	6/6	100	95.83	6.71

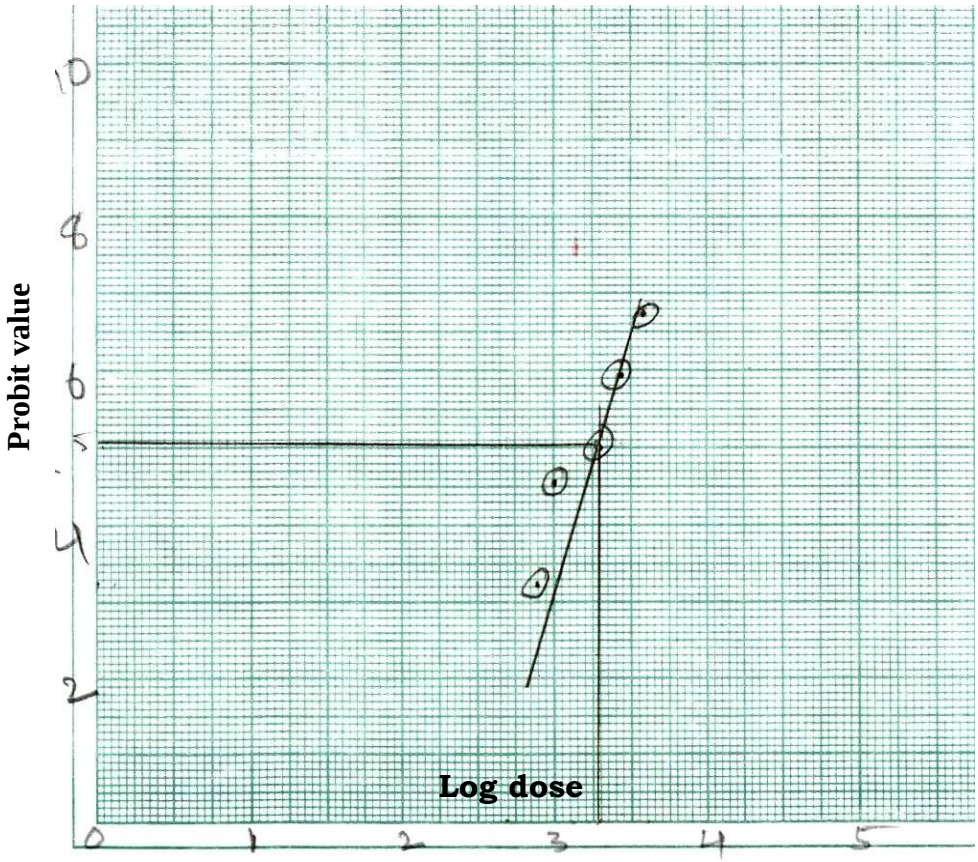
Correction:- $0\% = \frac{100 \times 0.25}{N} = 4.17$

$$100\% = 100\left(\frac{n - 0.25}{n}\right) = 95.83$$

LD_{50} = Antilog (3.29) = 1949.85 mg/kg.

LD_{50} DETERMINATION

Animal used : Albino rats
Weight : 150 – 200 gm
Route of administration: Intraperitoneal Route



LD_{50} dose = antilog of (3.29) = 1949.85 mg/kg

2. SUB- ACUTE TOXICITY STUDY IN ALBINO –RATS
Losartan Induced Sub- Acute Toxicity
15 Days Study
Body Weight

Animals Used	:	Albino Rats
Weight	:	150-200gm
Drug	:	Losartan
Route of Administration	:	Intra peritoneal route.
Dose of Losartan	:	1mg/kg (therapeutic dose)

1/10 the of the LD_{50} dose
(i) ⇒ 0.1mg/kg (therapeutic dose)
(ii) ⇒ 0.2 mg/kg (2 times of the therapeutic dose)
(iii) ⇒ 0.4 mg/kg (4time of the therapeutic dose)

TABULATE COLUMN – 2 (a)
Losartan Induced Sub- Acute Toxicity Study
15 Days Study
Body Weight (Control)

S. No.	Groups	Treatment Schedule	Mean body weight (Before treatment)	Mean body weight (After treatment)
1.	Control (6 animals)	Given on first day 0.1mg/kg Given on 6th day 0.2 mg/kg Given on 11th day 0.4 mg/kg	170 gm 169 gm 171 gm 172 gm 168 gm 169 gm	174 gm 176 gm 175 gm 177 gm 173 gm 174 gm
			Mean = 170 gm	Mean = 175 gm

TABULATE COLUMN – 2 (b)
Losartan Induced Sub – Acute Toxicity Study
15 Days Study
Body Weight (Losartan Treated Animals)

S. NO.	Groups	Treatment Schedule	Mean body weight (Before drug treatment)	Mean body weight (After treatment)
2.	Losartan Treated animals (6 animals)	Given on first day 0.1 mg /kg Given on first day 0.2 mg/kg Given on 11th day mg/kg	175 gm 174 gm 174gm 177gm 173gm 174 gm	179 gm 181 gm 180 gm 182 gm 179 gm 178 gm
			Mean = 175 gm	Mean = 180 gm

3. SUB- ACUTE TOXICITY STYDY IN ALBINO RATS TO MONITOR
a. Biochemical Changes

Tabulate Column

S. No.	Enzyme	Group	After 15 days the estimated level of Alkaline phosphatase	Meam ± SEM	Significance
1.	Alkaline Phosphatase	Test	149.0 KIU/L 152.3 KIU/L 154.8 KIU/L 150.0 KIU/L 153.6 KIU/L 150.0 KIU/L	151.3 ± 0.93	P < 0.001 't' = 23.2
2.	Alkaline Phosphatase	Control	120.4 KIU/L 125.8 KIU/L 123.6 KIU/L 121.1 KIU/L 123.4 KIU/L 124.5 KIU/L	122.67 ± 0.81	

1. ALKALINE PHASHATASE
For t = + 23.2 at 5degrees of freedom p < 0.001 therefore there is a highly significant increase in serum phophatase levels in rats.

Direct Bilirubin

S.No.	Enzyme	Test	After 15days the estimated level of bilirubin	Mean ± SEM	Significance
1.	Bilirubin Direct	Test	0.24 0.26 0.25 0.28 0.29 0.27	0.27 ± 0.79	p< 0.5 't' = 0.875
2.	Bilirubin Direct	Control	0.16 0.18 0.20 0.23 0.21 0.19	0.20 ± 0.01016	

2.a Direct Bilirubin

For t= 0.875 at 5 degrees of freedom at p< 0.5 therefore there is a significant decrease in serum direct biliurbin levels in rats.

Total Bilirubin

S. No.	Enzyme	Group	After 15 days the estimated level of bilirubin	Mean ± SEM	Significance
1.	Bilirubin Total	Test	0.49 mg % 0.53 mg % 0.47 mg % 0.50 mg % 0.51 mg % 0.54 mg %	0.51± 0.01016	p < 0.05 't' = 2.335
2.	Bilirubin Total	Control	0.35 mg % 0.39 mg % 0.37 mg % 0.40 mg % 0.41 mg % 0.43 mg %	0.39 ± 0.0116	

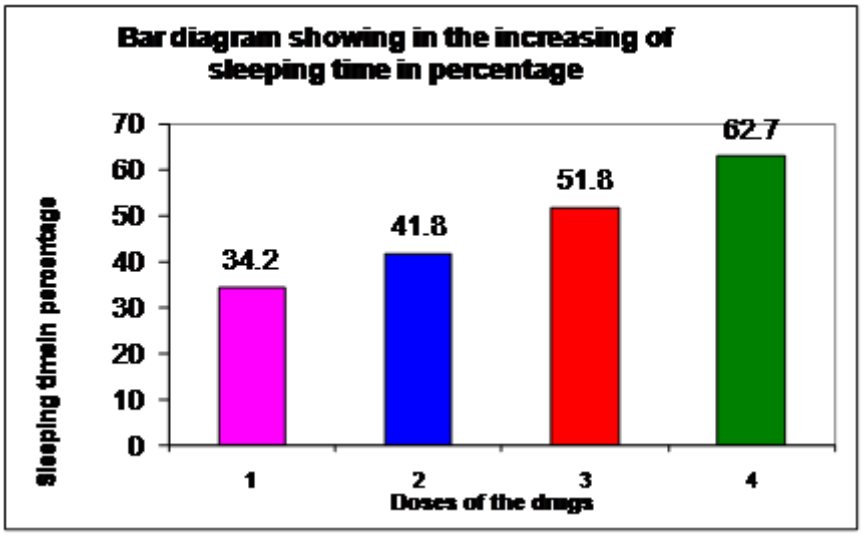
2.(b) For t = 2.335 of 5 degrees of freedom at p<0.05 their for is a significant increase in serum total bilirubin levels in rats.

Sleeping time with diazepam

Group	Drugs	Mean ± SEM
1	Control saline (0.1ml) + Diazepam (2 mg/100gm)	34.2 ± 0.75
2	Losartan (0.1 ml) + Diazepam (2mg/100gm)	41.8 ± 0.89
3	Losartan (0.2 ml) + Diazepam (2mg/100gm)	51.8 ± 0.914
4	Losartan (0.4 ml) + Diazepam (2mg/100gm)	62.7 ± 0.915

BAR DIAGRAM SHOWING IN THE INCREASING OF SLEEPING TIME IN PERCENTAGE

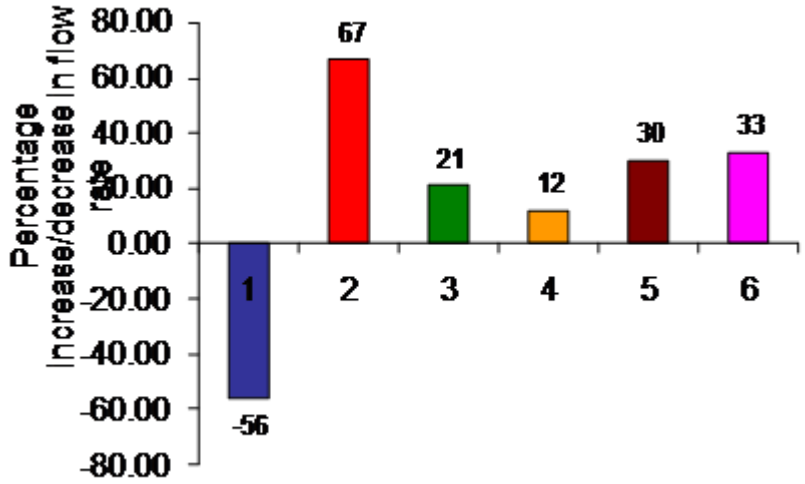
Animals : Albino rats
Control : 6 rats
Drug treated : 6 rats
Route of Administration : Intra peritoneal route



1 – Saline (0.1ml) + diazepam (2mg/100gm)
2 – Losartan (0.1ml) + diazepam (2mg/100gm)
3 – Losartan (0.2ml) + diazepam (2mg/100gm)
4 **– Losartan (0.4ml) + diazepam (2mg/100gm)**

PERCENTAGE INCREASE/DECREASE IN FLOW RATE BY DIFFERENT DRUGS IN PERFUSED BLOOD VESSELS OF FROG

Animal used : Forg
Weight : 150-200 gm
Physiological solution : Frog Ringer Solution
Drum speed : 0.25 mm/sec



1. Adrenaline – 10µG/ML
2. Acetylcholine - 50µG/Ml
3. Nitroglycerine – 1%
4. Losartan - 100µg /ml
5. Losartan - 200µg /ml
6. LOSARTAN - 400µG /ML

DISCUSSION

Losartan is one such selective antagonist of angio tensin II receptor introduced in market in 1998 for the treatment of hypertension[1]. But of late, there have been stray reports of Losartan an hepatotoxicity, cardiovascular toxicity fetal toxicity, renal toxicity. The present study is detailed investigation into the adverse effects and toxicity of Losartan in experimental animals.

Acute toxicity study
Acute toxicity study reveals that the LD_{50} dose of Losartan was found to be 2000 mg/kg.

Histopathological study
Control group of all animal showed normal histology.
The morphological feature of liver shows haemotological necrosis.
i.e. change in central vein and mild fatty damage, perilobular hepato cellular fatty damage, sinusoidal dilation.
Losartan does not appear to be nephrotoxic, since it dos not cause any morphological changes in the kidney.

Sub-acute toxicity study
Determination of the toxicity potential of a drug is very important before a drug is used in human. Though Losartan has came into market after its usual toxicity testing there are some recent clinical reports about is hepatotoxicity.

Hence conducted 15 days sub-acute toxicity studies in albino rats using 3 different doses of Losartan administered intra peritonially on a daily dosing schedule.

Drug-interaction studies
The sleeping time of diazepam with Losartan is increased in the drug treated group compared with that of control group due to the enzyme inhibition. The sleeping time is increased it reveals with the pharmaco kinetic study.

Effect of Losartan on isolated frog heart
The normal recording of the heart was recorded with a heart rate of 68. The Losartan in doses of 5µ, 10µ and 40µ all produced cardiac arrhythmia due to myocardial stimulation. The Losartan in a dose of 40µ was repeated after proranolol (20µ). The arrhythnia produced by Losartan could not be controlled by propranolol (refer graph). Therefore it implies that Losartan's stimulant effect is not mediated through β receptor and may be it directly stimulate the myocardiam so as to produce arrhythmia.

CONCLUSION

After the introduction of any new drug in the market we keep hearing about various adverse effects reported from different parts of the world during post marketing surveillance. A thorough experimental toxicological study of the same drug with experimental animals may throw light upon the possible ADRS likely to develop during human use in clinical practice.

So the investigator felt this kind of work could be encouraged as a part of pharmacological research.
The author is firmly convinced that the Losartan if exceeded in dose may lead to toxicities particularly haepatoxicity.

Moreover the drug inhibits mycrosomal enzymes. Therefore we must be careful when prescribing this drug with other drugs in order to avoid drug interaction phenomenon involving microsomal enzymes.

It has been proved the drug has produces cardiac arrhythmia in excess dose and upon repeated administration and Losartan is likely to produce vasodilatory effect also which is one of the criteria for its antihypertensive effects.

The author has made a humble effort by this project work to bring it to the notice of the clinical people about the various adverse reaction organ toxicities produced by Losartan and thereby promoting pharmaco vigilance in clinical scenerio.

REFERENCES

[1] Smith RD, Chiu AT, Wong PC, Herblin WF, Timmermans P. *Annu Rev Pharmacol Toxicol*. **1992**; 32: 135-165.
[2] Timmermans P, Benfield P, Chiu At, Herblin WF, Wong PC, Smith RD. *Am J Hypertens*. **1992**; 5 (Part 2): 221S-235S.
[3] Siegl PS. *J Hypertens*. **1993**; 11(suppl 3): S19-S22.
[4] Christen Y, Waeber B, Nussberger J, Porchet M, Borland N, Lee R, Maggon K, Shum L, Timmermans P, Burnner H. *Circulation*. **1991**; 83: 1333-1342.
[5] Munafo A, Christen Y, Nussberger J, Shum L, Borland M, Lee R, Waeber B, Biollaz J, Brunner H. *Clin Pharmacol Ther*. **1992**; 51: 513-521.
[6] Goldberg M, Tanaka W, BArchowsky A, Bradstreet T, McCrea J, Lo M-W, McWilliams E, Bjornsson T. *Hypertension*. **1993**; 21: 704-713.
[7] Cockcroft JR, Sciberras DG, Goldberg MR, Ritter JM. *J. Cardiovasc Pharmacol*. **1993**; 22: 579-584.
[8] Nelson E, Merrill D, Sweet C, Bradstreet T, Panebianco, Byyny R, Herman T, Lassester K, Levy B, Lewis G, *J. Hypertens*. **1991**; 9 (suppl 6) : S468-S469.
[9] Weber MA. *Am J Hypertens*. **1992**; 5