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A STUDY ON THE EFFECT OF LOSARTAN ON PHARMACOKINETIC AND ANTIDEPRESSANT ACTIVITY OF FLUOXETINE.

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ABSTRACT

Depression and hypertension are managed clinically by administering Number of drugs for longer duration. The studies have documented three- fold higher frequency of major depression in patients treated for hypertension extant. These two disorders are long-term disorders, treated with different drugs and there is a chance that the interaction between these drugs may appear which affects the management of single disorder or both disorders. We had studied the effect of losartan treatment on pharmacokinetic parameters and antidepressant activity of fluoxetine. The metabolic pathway for the concerned two drugs losartan and fluoxetine are by the same enzyme, CYP2C9. Healthy male albino rabbits were used to study the effect of losartan on pharmacokinetic parameters of fluoxetine followed by antidepressant activity to confirm the results. The concentration of fluoxetine in serum was estimated by HPLC and antidepressant activity was studied using despair swim test and serotonin syndrome in healthy albino rats. The serum concentration of fluoxetine was found significantly increased after losartan treatment for 7 days. The pharmacokinetic parameters like AUC, AUMC, Tmax, Cmax, $t_{1/2}$ and MRT of fluoxetine showed changes after losartan treatment for 7 days in healthy albino rabbits. Losartan treatment for one week exhibited significantly decreased in immobility time of fluoxetine tested by despair swim test and the severity of forepaw treading was significantly increased when tested in healthy rats. The

results revealed that the drug-drug interaction between fluoxetine and losartan could be due to strong protein binding property of both drugs and due to enzyme inhibition by CYP2C9.

KEYWORDS: Fluoxetine; Losartan; Depression; Hypertension, pharmacokinetics and despair swim test.

INTRODUCTION

A modification on the effect of a drug when administered with another drug this effect may be an increase or a decrease in the action of either substance or it may be an adverse effect that is not normally associated with either drug. Drug-drug interactions may occur when more than one therapeutic agent are administered in a patient to treat a single ailment or multiple ailments. The concomitant use of multiple drugs is often desired to obtain a therapeutic objective or to treat co-existing ailments. Simultaneous use of several therapeutic agents may lead to drug-drug interactions, results in altered patient's response to therapy which may be seen by enhanced or diminished effects of one or both of the drugs or the appearance of a new effect which is not seen with either drug alone. There are several diseases which require lifetime treatment for their management such as hypertension and diabetes. Patients with such diseases are often prescribed with multiple drugs for the treatment of other coexisting diseases, which might be either for a short period of time or lifelong. So, while prescribing medication it is important to determine the incidence and frequency of occurrence of drug interactions, which shows serious implications in hospitalized patients. In addition, it is also important to find out agents that are most likely to produce hazardous interactions. In this present study, an attempted has been made to find out the possibilities of occurrences of interactions between simultaneously used drugs prescribed for treatment of the two diseases namely; hypertension and depression which may co-exist and require chronic treatment.[1-6]

Hypertension or high blood pressure is a condition in which the force of blood against the artery walls is too strong. When blood pressure remains too high for many years, it can have deleterious effect on several parts of the body, the brain, heart, arteries and kidneys. Hypertension is sustained elevation of resting systolic blood pressure (BP) (= 140 mm Hg), diastolic BP (= 90 mm Hg) or both.[7]

Losartan is an angiotensin II (AT_1) receptor antagonist antihypertensive which acts by blocking the actions of the angiotensin II of renin-angiotensin-aldosterone system. Losartan potently and selectively inhibit, both in vivo and in vitro, most of biological effects of Angiotensin II, including angiotensin II-induced (1) contraction of vascular smooth muscle;

(2) rapid pressor response; (3) slow pressor response; (4) thirst; (5) vasopressin release; (6) aldosterone secretion; (7) release of adrenal catecholamines; (8) enhancement of noradrenergic neurotransmitter; (9) increase in sympathetic tone; (10) change in renal function; (11) cellular hypertrophy and hyperplasia.

Worldwide hypertension is estimated to cause 7.1 million premature deaths and 4.5% of disease burden (64 million disability adjusted life years). Hypertension plays a major etiologic role in the development of CVD, ischemic heart disease, cardiac and renal failure. Treating hypertension has been associated with about a 40% reduction in the risk of stroke and about a 15% reduction in risk of myocardial infarction.[8-9]

Depression is an extremely common psychiatric condition, about which a variety of neurochemical theories exist and for which a corresponding variety of drug are used in treatment. At any given moment, about 5 to 6% of the population is depressed (point prevalence), and an estimated 10% of population may become depressed during their lives (life time prevalence).The symptoms of depression are often subtle and unrecognized both by patient and physicians. Major depressive disorder has a lifetime prevalence of approximately 9.15% and perhaps as high as 20% in women. The mean age of onset is 35-40 years, although onset can be at any age. There are specific correlations with socioeconomic stress. In this present study possible interaction between an antihypertensive drug (losartan) and antidepressant drug (fluoxetine) was determined.[10-11]

Fluoxetine is a selective serotonin reuptake inhibitor (SSRI) and prescribed for the treatment of depression. Fluoxetine acts by desensitization of 5-HT1A somatodendritic receptors and 5HT1B nerve terminal auto receptors. However, there is no any literature regarding interactions between losartan and fluoxetine has been reported.[12]

Compounds which stimulate serotonin receptors or which increases dramatically the serotonergic transmission in the CNS cause a series of behavioral changes in rats which is called the serotonin syndrome such as head weaving, increased locomotion, forepaw treading, tremor, hind limb abduction, flat posture and lower lip retraction. With increasing knowledge about the subtypes of serotonin receptors these symptoms were defined to be associated with 5-HT receptors and their specific agonists. The behavioral motor syndrome (5-HT syndrome) can be elicited by injecting, the serotonin precursor. The serotonin syndrome in rats has been used to study the interaction of drugs with central 5-HT system of rats. It is also used for the

screening of the psychoactive drugs and this method offers several advantages like this is fast, require no elaborate equipment and provide information on CNS permeability.[13]

The main objective of the present study was to assess the effect of losartan on pharmacokinetic and antidepressant activity of fluoxetine in healthy rat, mice and rabbits and also to suggest the alterations in the dose and frequency of administration of fluoxetine, if necessary. Interaction between fluoxetine and losartan could be due to their strong protein binding and by similar metabolic pathway. When both of the drugs are given together fluoxetine dose should be decreased.

MATERIAL AND METHODS

Chemical used: Pure sample of Fluoxetine and losartan was obtained as a gift sample from Time Pharma, Nepal. Surgical spirit, Methanol, Acetonitrile were procured from S.D Fine chemicals, Mumbai, India. All the chemicals used were of analytical grade.

Animal used: Rabbits (2-2.5 kg), rats (150-200 gm), mice (18-22 gm). All animal used were male sex and albino species.

Ethical approval: The study protocol was approved by Institutional Animal Ethics Committee (IAEC), Mallige College of Pharmacy, Bangalore.

Reg. no. 1610/RO/C/12/CPCSEA

HOUSING OF EXPERIMENTAL ANIMALS

Rabbits were housed in stainless steel cages with a fenestrated Floor to allow faeces to drop through into a pan and were provided with regular rabbit chow. Rats are housed in separate clean cages. The bedding material of the cages rats were removed and replaced thrice a week with fresh materials as often as necessary to keep the animals clean and dry. The animals were provided with distilled water ad libitum throughout the experiment. The rats were fed with standard pelleted diet. The animals were acclimatized to standard laboratory conditions of temperature (25 ± 3°) and maintained on 12:12 h natural light: dark cycle. The animals were maintained under standard conditions in an animal house approved by Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA).

EXPERIMENTAL PROCEDURE

Effect of Losartan treatment on pharmacokinetic parameters of Fluoxetine in Healthy *albino* rabbits: Four male *albino rabbits* were taken and marked suitably. Rabbits were fasted for 18 h before commencing the experiment and the blood was collected (at '0' h) before the administration of fluoxetine. Later all the rabbits received fluoxetine (10 mg/kg) solution orally, the time of administration was noted. Blood samples were collected thereafter at prefixed time intervals i.e. 0, 2, 4, 8, 16 and 24 h after dosing. Blood samples were collected in tube, kept a side and centrifuge for 15-20 min at 3000 rpm to collect serum. Serum samples were stored at 2-8° for analysis. After blood collection animals were left for a washout period of 15 days with normal diet. The next part of this experiment was conducted on the same group of animals. All the rabbits received losartan (10 mg/kg) orally once a day for one week. On the 7th day, 6 h after administration of the drug, the rabbits were fasted for 18 h. On the 8th day, losartan (10 mg/kg) was administered orally to all the animals; the time of administration was noted. After 60 min of losartan administration, fluoxetine (10 mg/kg) was given orally. Blood samples were collected in a blood collection tube at prefixed time intervals i.e. 0, 2^{nd}, 4^{th}, 8^{th}, 16^{th} and 24^{th} h after fluoxetine dosing, serum was separated from blood and stored at 2-8° for analysis. The serum concentration of fluoxetine was estimated by High Performance Liquid Chromatography method.[14-15]

Effect of losartan treatment on antidepressant activity of fluoxetine in healthy *albino* rat by despair swim test: Six Male *albino rats* were brought to the laboratory one day before the experiment and were housed separately in cages with free access to food and water. Rats are individually forced to swim inside a vertical Plexiglas cylinder (height: 40 cm; diameter: 18 cm) containing 15 cm of water maintained at 25°. Rats placed in the cylinder for the first time are initially highly active, vigorously swimming in circles, trying to climb the wall or diving to the bottom. After 2-3 min activity begins to subside and to be interspersed with phases of immobility or floating of increasing length. After 5-6 min immobility reaches a plateau where the rats remain immobile for approximately 80% of the time. After 15 min in the water the rats are removed and allowed to dry for 1 h, later and the total duration of immobility is measured during a 5 min test. An animal is judged to be immobile whenever it remains floating passively in the water in a slightly hunched but upright position, its nose just above the surface. In the first part of experiment, animals were administered with fluoxetine (10 mg/kg) in a heated enclosure (32°) before being returned to their home cages. They are again placed in the cylinder 24 h and the total duration of immobility is measured during a 5 min

test. An animal is judged to be immobile whenever it remains floating passively in the water in a slightly hunched but upright position, its nose just above the surface. In the next part of the experiment, the same group of animals after a gap of 15 days was administered with losartan (10 mg/kg) once a day for one week. On the 8th day, losartan (10 mg/kg, p.o.) were administered to all the animals, and the time of administration was noted. After 60 min of losartan administration, fluoxetine (10 mg/kg) was administered, the test was repeated and the total duration of immobility for duration of 5 min was measured at 0,2nd, 4th, 8th, 16th and 24th h after fluoxetine administration.[16]

SEROTONIN SYNDROME

Forepaw Treading Test: Six male *albino rats* weighing between 160-180 grams were selected and housed in cage with free access to food and water. In the first part of experiment, rats were administered with fluoxetine (10 mg/kg, p.o.). The time of the drug administration was noted for all the animals. After 30 minutes of fluoxetine administration, 5-hydroxytryptophan (5-HTP) (25mg/kg, p.o.) was administered to all the rats. Each rat was scored during 0-15, 15-30, 30-45, and 45-60, minute after the oral administration. The severity of the symptoms were scored as following scale, forepaw treading (0=absent; 1=weak; 2=continuous). All the rats were left for washout period of 15 days.

In the next part of the experiment, the same animals after a gap of 15 days were administered with losartan (10 mg/kg, p.o.) once a day for one week. On the 8th day, losartan (10mg/kg, p.o.) was administered to all the animals, and the time of administration was noted. After 60 minutes of losartan administration, fluoxetine (10 mg/kg, p.o.) was administered. Again after 30 minutes of i.e. administration, 5-hydroxytryptophan (25mg/kg, p.o.) was administered to all the rats. Severity of symptoms was scored as mentioned earlier. The results obtained are tabulated in the table 4, depicted in figure 5.

Statistical Evaluation: The data of methods are expressed as mean ± SEM for each treatment group. The data obtained from each response measures were subjected to student 't' test using parametric statistics, Graph Pad Prism trial version 6.01. A value of $P < 0.05$ was considered statistically significant.

RESULTS AND DISCUSSION

Effect of losartan on pharmacokinetic parameters of fluoxetine: The serum concentration of fluoxetine before and after the treatment of losartan is tabulated in **table 1**. The serum

concentration of fluoxetine at 2nd, 4th, 8th, 16th and 24th hour was increases after losartan treatment. The peak concentration was observed at 4th hour i.e, 56.47 ng/ml and declining at 8th, 16th and 24th hour, which is graphically represented in **figure 1.** The pharmacokinetic parameters are tabulated in **table 2.** It revealed that AUC and AUMC of fluoxetine was changed after losartan treatment. The C_{max}, AUC and AUMC of fluoxetine are increased due to losartan treatment, which is graphically represented in **figure 2** and **3.** These results revealed the absorption of fluoxetine is increased by losartan treatment.

The losartan treatment for one week significantly increased the serum concentration of fluoxetine at all hour. The peak concentration (C_{max}) of fluoxetine is increased from 40.14ng/ml to 56.47ng/ml before and after treatment rabbit with losartan. The time of peak concentration (T_{max}) did not change.

Effect of losartan treatment on antidepressant activity of fluoxetine by despair swim test in healthy albino rats: The results are shown in **Table 3**, indicates that fluoxetine exhibited immobility time of 65.67 seconds at the initial state i.e. 0 hour followed by 58, 37.5, 42.5, 53.67and 54.83 seconds at 2th, 4th, 8th, 16th, and 24th hour respectively. The maximum effect is shown in 4th hour i.e. 37.5 seconds after fluoxetine treatment only. Simultaneously effect was decreased after 4th hour i.e. 42.5, 53.67, 54.83 second at 8th, 16th and 24th hour respectively. Immobility time show significant changes during 4th and 8th hour, but at 0, 16th and at 24th hour no show significant changes occurs. These results confirm their antidepressant activities are tested in this animal model.

Losartan treatment for one week decreased the immobility time in healthy albino rats significantly at 0, 2nd, 4th, 8th, 16th and 24th hour. The immobility time is reduced in all hours. But significant decrease is shown in 4th and 8th hour. However at 0 and 2nd hour did not showed significant difference. The least immobility time is seen in 4th hour i.e. 32.67 seconds, which is graphically represented in **figure 4.**

Effect of losartan treatment on anti-depressant activity of fluoxetine by serotonin syndrome test in healthy albino rats: Data shown in the **Table 4,** represent that fluoxetine treatment on rats with serotonin precursor has a significant effect on fore paw treading. Rats treated with fluoxetine only scored 0.5, 0.83, 1.17 and 1.5 at time 0-15, 15-30, 30-45 and 45-60 minutes respectively. The maximum score was obtained at 30-45 minutes i.e. 1.5. But at

initial stage there was low symptom of fore paw treading and was increased after 15 minutes till 1 hour.

After the administration of losartan along with fluoxetine and serotonin precursor difference in score was found. Difference in score was 1.17, 1.5, 1.83 and 1.83 at time 0-15, 15-30, 30-45 and 45-60 minutes respectively. The significant difference in score was found during the period of 45-60 minutes, which is graphically represented in **figure 5.**

Serotonin syndrome is associated with increased serotonergic activity in the central nervous system (CNS). Serotonin syndrome is a potentially fatal complication of serotonergic drug therapy. Usually, serotonin syndrome occurs with the concomitant use of two serotonergic drugs. In the present study losartan significantly potentiated the serotonin syndrome of fluoxetine.

Table 1 Data showing the serum concentration of fluoxetine before and after losartan treatment in healthy albino rabbits

	Serum concentration of fluoxetine in ng/ml		
S. No.	**Time in Hr**	**Fluoxetine (10 mg/kg, p.o.)**	**Fluoxetine+Losartan (10mg/kg+10mg/kg, p.o.)**
1	0	_	_
2	2	32.91 ± 2.05	44.90 ± 4.16 *
3	4	40.14 ± 1.27	56.47 ± 3.9 *
4	8	14.05 ± 0.62	17.38 ± 0.75 **
5	16	10.63 ± 0.71	15.39 ± 0.44 **
6	24	8.95 ± 0.59	12.61 ± 0.43 **

Number of rabbit per group (N) =4
Values are expressed as Mean± SEM
*P<0.05
**P<0.01

Table 2 Data showing the effect of losartan treatment on pharmacokinetic parameters of fluoxetine in healthy albino rabbits.

Pharmacokinetic parameters	**Fluoxetine**	**Fluoxetine + Losartan**
AUC_{0-t} (ng/ml/hr)	391.33	537.05
$AUMC_{0-t}$ (ng/ml/hr)	4882.82	6925.33
$t_{1/2}$ (hr)	24.58	34.56
C_{max} (ng/ml/hr)	40.14	56.47
T_{max} (hr)	4	4
MRT (hr)	33.52	45.78

AUC_{0-t} Area under curve

$AUMC_{0-t}$ Arear under first order moment curve.

$t_{1/2}$ Terminal Half life

C_{max} Concentration maximum

T_{max} Time of concentration maximum

MRT Mean residential time

Table 3 Data showing the immobility of fluoxetine before and after losartan treatment in healthy albino rats using despair swim test.

S. No.	Time in hours	Immobility time (sec) in 5 minutes test	
		Drug treatment	
		Fluoxetine (10 mg/kg) p.o.	Fluoxetine+Losartan (10 mg/kg) p.o.
1	0	65.67 ± 1.15	62.67 ± 0.99 *
2	2	58 ± 1.53	51.33 ± 0.95 *
3	4	37.5 ± 0.99	32.67 ± 1.23 ***
4	8	42.5 ± 1.18	38.33 ± 0.67 *
5	16	53.67 ± 0.80	48.50 ± 0.76 **
6	24	54.83 ± 1.20	46.17 ± 1.25 **

Number of rat per group (N) = 6

Values are expressed as Mean ± SEM

*P<0.05

**P<0.01

***P<0.001

Table 4 Data showing the forepaw treading score of fluoxetine before and after losartan treatment in healthy albino rats by serotonin syndrome test.

Forepaw treading score in different time interval			
S. No.	Time interval in min	Fluoxetine (10mg/kg)p.o.+5HTP (25mg/kg)i.p.	Fluoxetine+Losartan(10mg/kg)p.o. +5HTP (25mg/kg)i.p.
1	15	0.50 ± 0.22	1.17 ± 0.17 *
2	30	0.83 ± 0.17	1.50 ± 0.22 *
3	45	1.17 ± 0.17	1.83 ± 0.17 *
4	60	1.50 ± 0.23	1.83 ± 0.17

Number of rat per group (N) =6

Values are expressed as Mean± SEM

*P<0.05

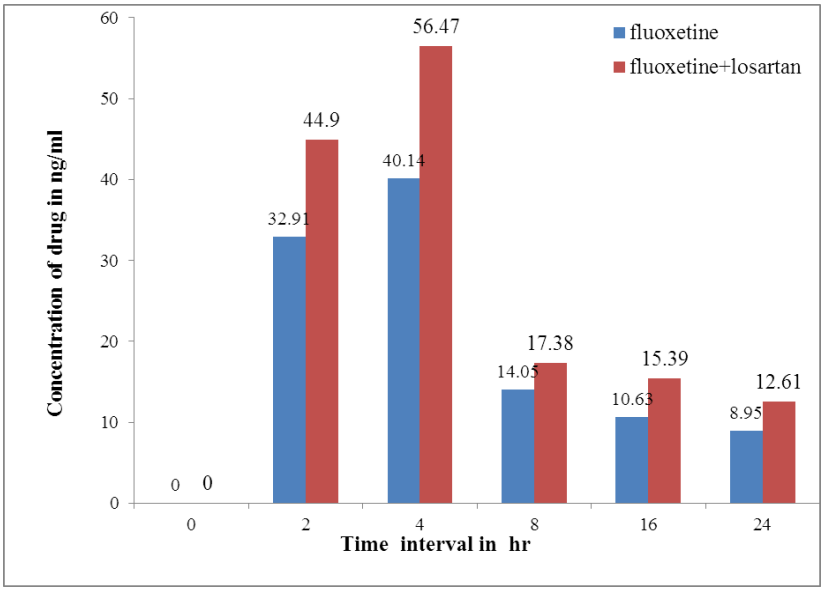

Figure 1 Graphical representation showing the serum concentration of fluoxetine before and after losartan treatment in healthy albino rabbits in different time interval.

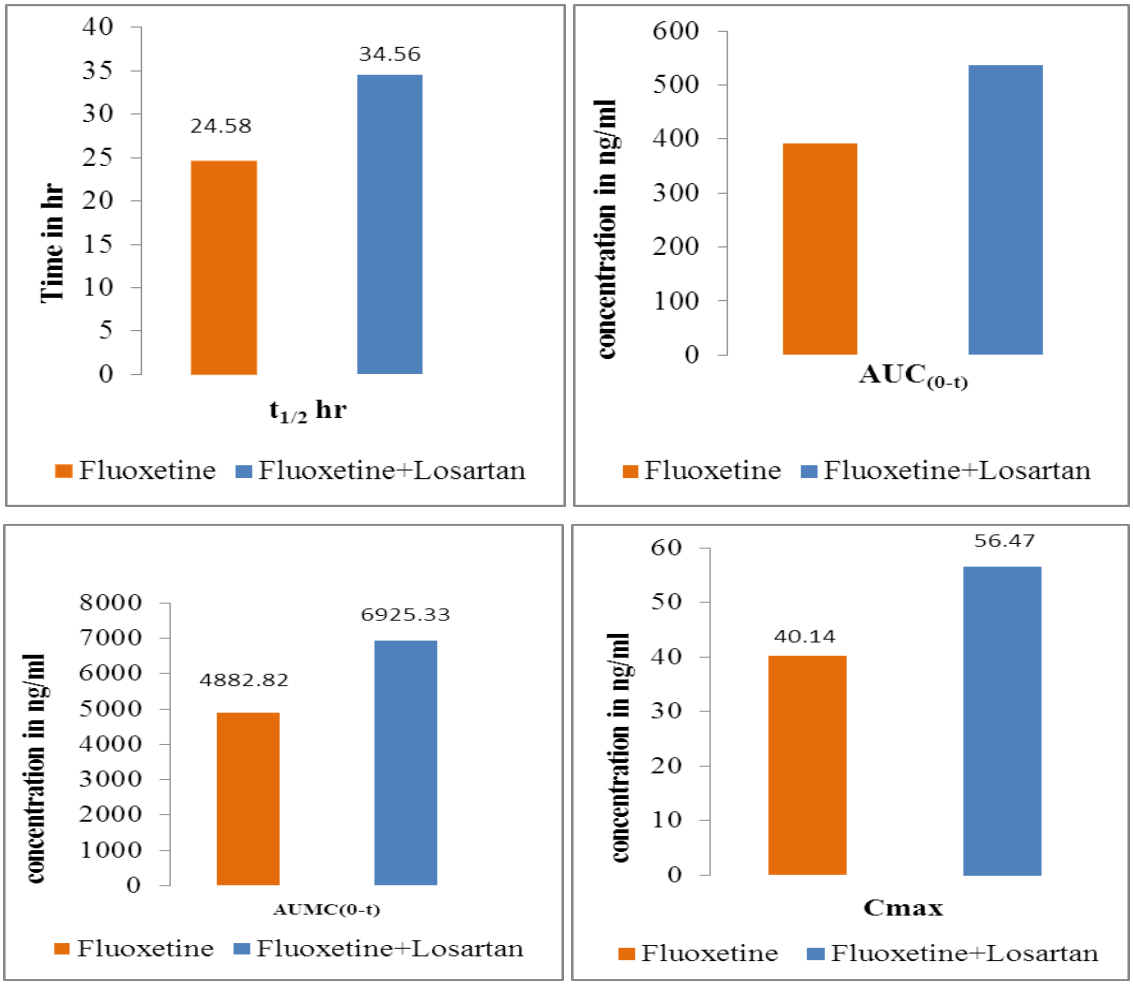

Figure 2 Graphical representation of the effect of Losartan on the pharmacokinetic parameters of Fluoxetine.

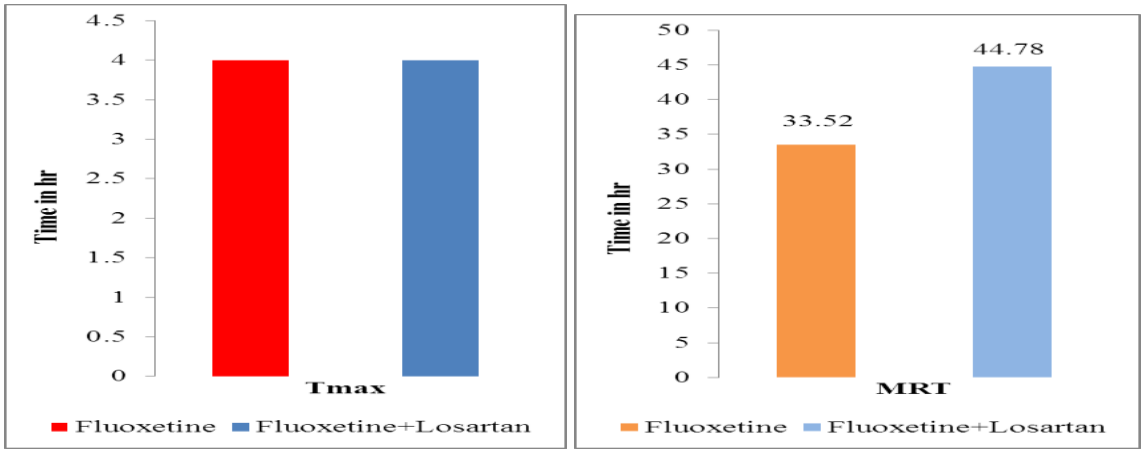

Figure 3 Graphical representation showing theTmax and MRT of fluoxetine before and after losartan treatment in healthy albino rabbits.

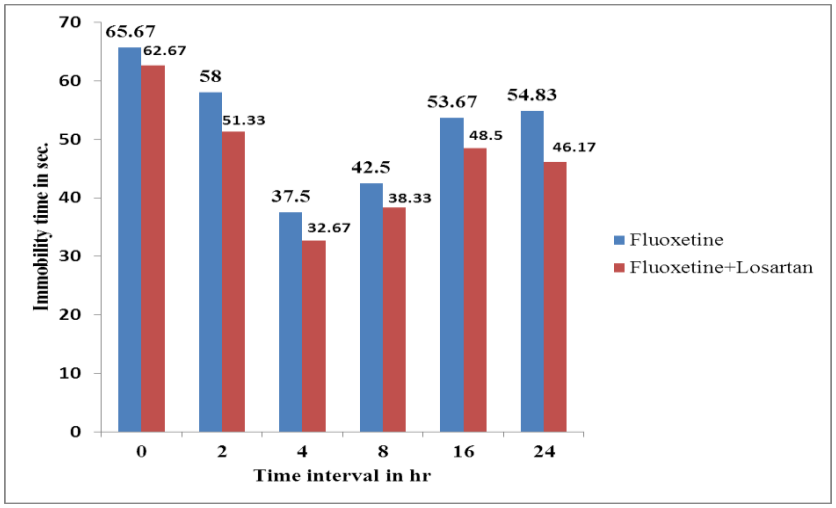

Figure 4 Graphical representation showing the effect on immobility time of fluoxetine treatment rats before and after losartan treatment by despair swim test.

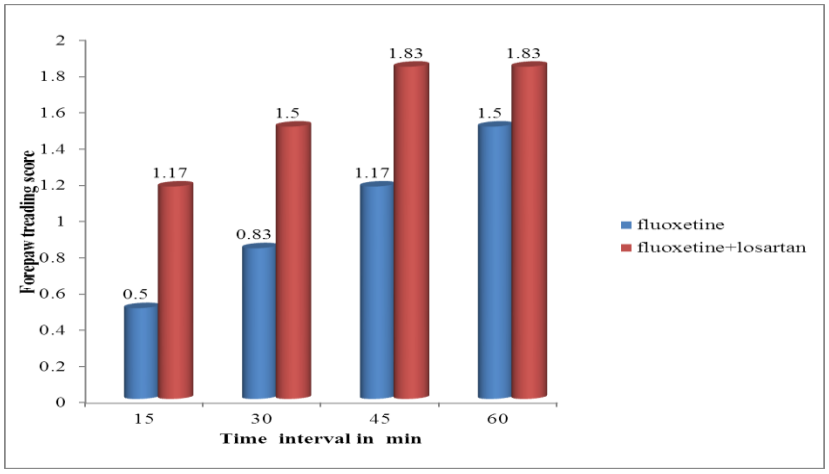

Figure 5 Graphical representation showing the effect on forepaw treading score in fluoxetine treatment rats by serotonin syndrome before and after losartan treatment.

CONCLUSION

The effect of losartan on pharmacokinetic and antidepressant activity of fluoxetine on healthy *albino* rabbits and rats was studied. The results obtained from the present study suggest that there is an interaction when losartan is co-administered with fluoxetine. The pharmacokinetic parameters of fluoxetine were significantly changed in the rabbits pre- treated with losartan. Significant changes in immobility time and severity in forepaw treading score of fluoxetine were also observed in rats pre-treated with losartan.

These changes in the pharmacokinetic and antidepressant activity may be due to their potentiating effect in inhibiting serotonin uptake in brain synaptosomes and strong protein binding property of fluoxetine and losartan, or may be due to its inhibitory effect on CYP2C9 isoenzyme.

The exact mechanism of this interaction cannot be predicted at this stage. Furthermore research into the effect of losartan on serotonin concentration in brain synaptosomes, protein binding and the chronic treatment of fluoxetine is required for predicting the molecular mechanism behind interaction.

The consumption of antidepressant and angiotensin II antagonist is common among patient suffering from hypertension and depression. The interfering effects of losartan and fluoxetine must be cautiously considered if patient is consuming losartan and fluoxetine together.

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