IJPSR (2014), Vol. 5, Issue 2 **(Research Article)**

INTERNATIONAL JOURNAL OF PHARMACEUTICAL SCIENCES AND RESEARCH

Received on 14 September, 2013; received in revised form, 04 November, 2013; accepted, 10 January, 2014; published 01 February, 2014

STUDY OF ANTIULCER ACTIVITY OF ANGIOTENSIN RECEPTOR ANTAGONISTS IN EXPERIMENTALLY INDUCED GASTRIC ULCERS IN RATS

Suvarna S. Rathod *[1], Vijay M. Motghare [1] and Vinod S. Deshmukh [2]

Department of Pharmacology, B.J. Medical College [1], Pune, Maharashtra, India
Department of Pharmacology, S.R.T.R. Medical College [2], Ambajogai, Maharashtra, India

Keywords:

peptic ulcer, angiotensin receptor antagonists, antiulcer activity, gastroprotective

Correspondence to Author:

Dr. Suvarna S Rathod

Flat no 17, Laxmikantam Apartment, Tirupati Nagar, Warje, Pune-411052, Maharashtra, India

E-mail: dr.su16@gmail.com

ABSTRACT:

Background: Peptic ulcer is one of the common diseases affecting the mankind. Many studies reveal co-occurrence of both peptic ulcer and hypertension in humans. So, it might be beneficial to choose an optimal drug that will target both these conditions. The role of renin-angiotensin system in the pathogenesis of peptic ulcer is well known. AT receptor antagonists which prevent binding of AT-II to AT_1 receptors, have been thought to be implicated in protection of gastric mucosa against development of ulcers.
Objective: To study the antiulcer activity of telmisartan and candesartan in gastric ulcers induced by pylorus ligation method and indomethacin induced ulcer method in albino rats.
Material and methods: Antiulcer activity of telmisartan and candesartan was tested against the standard antiulcer drug ranitidine, using two experimental gastric ulcer models; pylorus ligation method and indomethacin induced ulcer method using six albino rats for each drug and each ulcer model. Parameters used for studying the antiulcer activity were free and total acidity of the gastric contents in pylorus ligation method and ulcer index in both the models.
Conclusion: The present study provides evidence that AT-II AT_1 antagonists play a role in protection against gastric ulcers in both the experimental models. This concept may provide a new path to develop important therapies for effectively treating two concurrently occurring diseases i.e., hypertension and peptic ulcer, with a single drug.

INTRODUCTION: Peptic ulcer is one of the commonest diseases affecting the mankind. They are so common in industrialized nations that, they represent, 'stigmata of civilization' [1]. Currently available antiulcer medications provide only pain relief and healing of ulcers but no drug prevents ulcer recurrence. So the search for newer medications which will effectively reduce complications and prevent relapse is greatly needed. Hypertension is also a very common entity. Some epidemiological studies reveal co-occurrence of both peptic ulcer and hypertension in humans [2].

Sonnenberg *et al* (1988) reported that gastric ulcer coincided more frequently with cardiovascular diseases related to hypertension and both share a common etiological factor [3].

So, it may be beneficial to choose an optimal drug that will target both these conditions.

QUICK RESPONSE CODE

DOI: 10.13040/IJPSR.0975-8232.5(2).502-07

Article can be accessed online on: www.ijpsr.com

DOI link: http://dx.doi.org/10.13040/IJPSR.0975-8232.5(2).502-07

Renin- angiotensin system (RAS) is involved in homeostatic control of arterial pressure, tissue perfusion and extracellular volume. Angiotensin (AT) II is the key component in various physiological and pathological processes mediated by RAS. The components of RAS; angiotensinogen, renin and angiotensin converting enzyme (ACE) from which AT-II is formed, are present not only in systemic circulation but also in the gastric wall [4].

This suggests that AT-II is formed locally within the stomach to control the gastroduodenal blood flow i.e., it constricts the gastric vasculature [5]. It has also been observed that AT_1 receptors are expressed in endothelium of arteries located in gastric mucosa, muscularis mucosa and larger arteries in submucosa. The role of renin-angiotensin system in the pathogenesis of peptic ulcer is well known [6].

Stress is one of the etiological factors for pathogenesis of peptic ulcer. AT-II is a stress hormone [7] whose levels get increased in plasma and tissues like brain, kidneys, liver, including stomach during stress. This generates reactive oxygen species (ROS) which cause cell inflammation and damage. During stress, there occurs increased expression of the proinflammatory cytokines; TNF-α and intracellular adhesion molecule-1 (ICAM-1) along with neutrophil infiltration and leukocyte migration in the gastric mucosa [8].

So, by virtue of its variety of effects including oxidative damage, inflammation, and impaired gastroduodenal blood flow; AT-II in involved in the pathogenesis of peptic ulcers. Therefore, AT receptor antagonists which prevent binding of AT-II to AT_1 receptors; have been thought to be implicated in protection of gastric mucosa against development of ulcers.

Studies have reported antiulcer effects of various AT-II AT_1 receptor antagonists in experimentally induced gastric ulcers in rats. A recent study has shown that telmisartan and candesartan significantly attenuated free and total acid output in a combined pylorus ligation with cold restraint stress method and pylorus ligation with indomethacin induced ulcer method. These drugs also decreased the gastric lesions in both the ulcer models [9]. During the literature search, no study was found evaluating the antiulcer effects of telmisartan and candesartan in isolated pylorus ligation induced ulcer method.

This finding was encouraging to conduct the present study using telmisartan and candesartan in order to evaluate their probable protective role in isolated pylorus ligation method and to further confirm their antiulcer role which is suggested by various studies in indomethacin induced ulcer method in albino rats.

MATERIALS AND METHODS: The experimental protocol was approved by Institutional Animal Ethics Committee, S.R.T.R Medical College, Ambajogai, Maharashtra, India.

Experimental animals used: Adult albino rats of either sex weighing 150-200gms were used. They were randomly allocated to different groups of six and placed in suitable cages with grating as the floor to avoid coprophagy. The rats in which coprophagy had occurred, were excluded from the study. The rats were allowed to adjust to the laboratory conditions such as light, temperature and noise before being subjected to the experiments. Prior to the experimentation, they were fasted for 24-48 hours allowing free access to water. In order to avoid the influence of diurnal variation, all the experiments were carried out at the same time of the day i.e., between 9am to 6pm.[10]

Drugs and chemicals: Telmisartan (Medley Pharmaceuticals, Jammu), candesartan (Ranbaxy Laboratories Ltd, Haryana), ranitidine (yarrow chem products laboratories Ltd, Mumbai), indomethacin (Cipla Ltd, Mumbai) carboxymethyl cellulose (CMC), phenolphthalein, Topfer's reagent, Tris buffer, pyrogallol, trichloroacetic acid, thiobarbituric acid, N-butanol.

Experimental Design:

a) **Pylorus Ligation-Induced Gastric Ulcer:** Albino rats weighing 150-200gms were housed in individual cages and fasted for 48 hours with free access to water. Care was taken to avoid coprophagy. Under light ether anesthesia, the abdomen was opened by a small midline incision below the

xiphoid process. Pyloric portion of the stomach was lifted out and ligated avoiding traction to the pylorus or damage to its blood supply. The stomach was replaced carefully and the abdominal wall was closed by interrupted sutures. The test drugs were administered orally, in a single dose, 30 minutes before pyloric ligation. The animals were deprived of food and water in postoperative period and sacrificed nineteen hours after operation. Stomachs were dissected out; contents drained into tubes and sent for determination of free and total acidity in the Department of Biochemistry in this institution. The stomachs were then cut open along the greater curvature and inner surface was examined for ulceration. The ulcer index was determined.[11]

b) **Estimation of free and total acidity in pylorus ligated rats:** Secretions from the stomach were collected individually in centrifuge tube. After centrifugation, clear supernatant fluid was taken for acid estimation. Free and total acidity was estimated by titration method by Hawk PB et al (1947).[12]

c) **Indomethacin Induced Gastric Ulcer:** Albino rats of either sex in a group of six were used. Animals were fasted for 48 hours. Indomethacin in a dose of 10mg/kg was given orally, in two doses at fifteen hours interval. The animals were sacrificed six hours after second dose of indomethacin. The test drugs were given 30 minutes before each dose of indomethacin. The control group received no drug treatment before noxious challenge. The stomachs were dissected and the ulcer index was calculated [10].

The ulcer index was calculated using the following equation [10];

$$\text{Ulcer index} = 10 / X$$

Where, X= Total mucosal area/ Total ulcerated area

Total ulcerated and mucosal area was calculated using ImageJ software [13].

Statistical Analysis: Statistical analysis was performed by one-way analysis of variance (ANOVA) followed by Bonferroni's multiple comparison test. The data were expressed as Mean ± S.E.M. p value <0.05 was considered as statistically significant. The data was analyzed by using "GraphPad Prism, version 6.00 for Windows, GraphPad software, San Diego California USA, www.graphpad.com."

RESULTS:

Effect of Telmisartan and Candesartan on Ulcer Index and Gastric Parameters in Pylorus Ligation Induced Ulcers: Table 1 shows that both the drugs produced significant reduction in ulcer index compared to control group. ($p < 0.05$). But, it was highly significantly less when compared to ranitidine ($p< 0.001$). Telmisartan and candesartan showed no statistically significant difference in reducing ulcer index when compared with each other. **(Fig. 3)**

Telmisartan was highly significant in reducing free as well as total acidity when compared to the control group ($p<0.01$ and $p<0.001$ respectively). Also, candesartan significantly reduced the free and total acidity when compared to control group ($p<0.05$). Effects of telmisartan and candesartan in reducing free as well as total acidity showed no statistically significant difference when compared with each other. **(Fig. 1 and Fig. 2.)**

TABLE 1: EFFECT OF ON ULCER INDEX AND GASTRIC PARAMETERS IN PYLORUS LIGATION INDUCED ULCERS

Sr. no.	Groups (n=6)	Gastric Ulcer Index	Free Acidity (mEq/l)	Total Acidity (mEq/l)
1	Control	0.40 ± 0.028	42.33 ± 2.654	74.67 ± 3.947
2	Ranitidine	0.053 ±0.013$^{***\#\#\#}$	11.45 ± 1.086$^{***\#\#\#}$	35.08 ±1.562$^{***+++a}$
3	Telmisartan	0.32 ± 0.013*	28.67 ± 2.275**	52.08 ± 2.043***
4	Candesartan	0.30 ± 0.012*	32.17 ± 2.880*	59.00 ± 4.747*

All values are mean ± S.E.M. (n=6). * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$ Vs control. # # # $p< 0.001$ Vs both telmisartan and candesartan. +++ $p< 0.001$ Vs candesartan. $p< 0.05$ when compared to telmisartan.

Effect of Telmisartan and Candesartan on Ulcer Index Indomethacin Induced Ulcers: Telmisartan and candesartan were highly significant in reducing ulcer index as compared to control group ($p < 0.001$). Effect of both telmisartan and candesartan was significantly less than ranitidine. While, reduction in ulcer index by telmisartan was significant when compared to that of candesartan. **(Fig. 4)**

TABLE 2: EFFECT ON ULCER INDEX IN INDOMETHACIN INDUCED ULCERS:

Sr. no.	Groups (n=6)	Gastric Ulcer Index
1	Control	0.59 ± 0.037
2	Ranitidine	0.07 ± 0.011$^{***+++a}$
3	Telmisartan	0.20 ± 0.021$^{***\#}$
4	Candesartan	0.30 ± 0.023***

All values are mean ± S.E.M. (n=6). *** p< 0.001 Vs control. # p< 0.05 Vs candesartan. +++ p<0.001 Vs candesartan. [a] p<0.05 Vs telmisartan

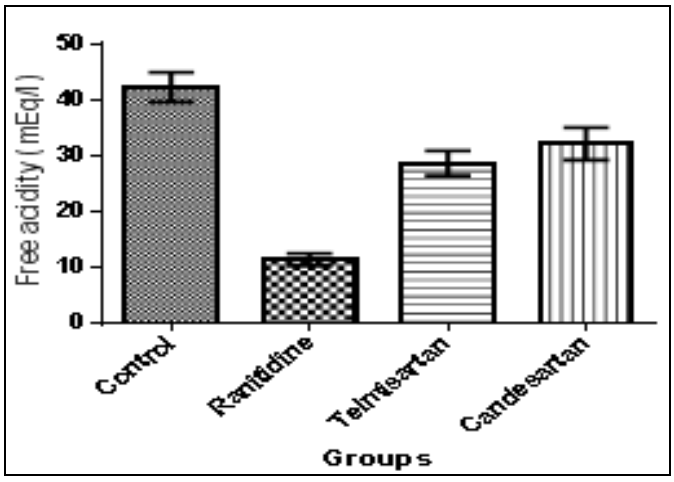

FIGURE 1: EFFECT OF TELMISARTAN AND CANDESARTAN ON FREE ACIDITY IN PYLORUS LIGATION METHOD

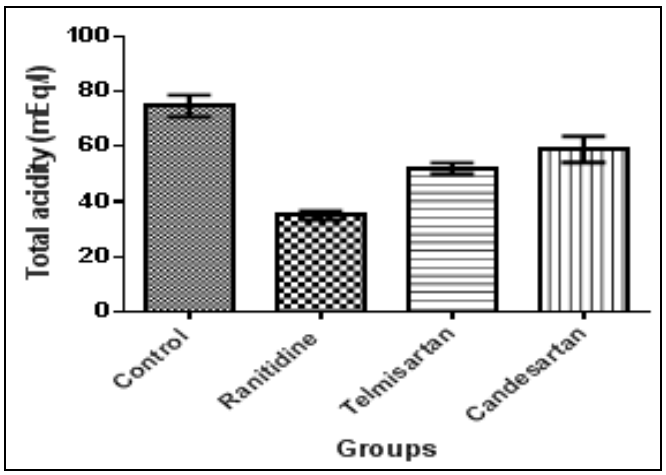

FIGURE 2: EFFECT OF TELMISARTAN AND CANDESARTAN ON TOTAL ACIDITY IN PYLORUS LIGATION METHOD

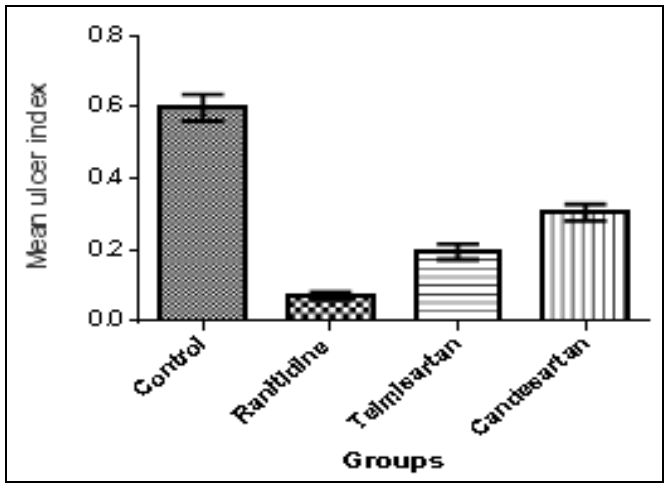

FIGURE 3: EFFECT OF TELMISARTAN AND CANDESARTAN ON ULCER INDEX IN INDOMETHACIN INDUCED ULCER METHOD

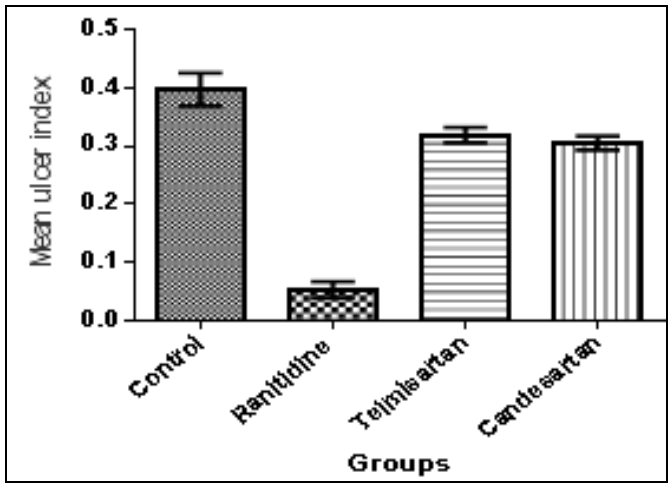

FIGURE 4: EFFECT OF TELMISARTAN AND CANDESARTAN ON ULCER INDEX IN PYLORUS LIGATION METHOD

DISCUSSION: The present study investigated the effect of two angiotensin receptor antagonists, telmisartan and candesartan on gastric secretion and gastric ulcer index using two experimental models i.e., pylorus ligation induced ulcer method and indomethacin induced ulcer method. This study helps in determining the role of AT-II AT_1 receptors in pathogenesis of peptic ulcers and probable therapeutic effect of angiotensin receptor antagonists in the treatment of peptic ulcer disease.

In pylorus ligated rats, there is accumulation of gastric acid and pepsin in the stomach leading to development of ulcers [14]. The probable mechanism of increased acid output in pylorus ligation method in rats with intact vagus, is that this acid response is elicited by vago-vagal reflexes activated by pressure receptors located in the pyloric gland area [15].

In present study, both telmisartan and candesartan showed significant antisecretory activity by decreasing free and total acidity and gastroprotective activity by significant reduction in ulcer index.

A recent study conducted using telmisartan and candesartan in combined indomethacin induced with pylorus ligation and CRS (cold restraint stress) with pylorus ligation method, showed that telmisartan and candesartan significantly reduced the ulcer indices in both models. While both the drugs showed significant antisecretory activity by reducing free and total acidity only in indomethacin induced ulcer model and not in CRS induced ulcers.[9] Moreover, during the literature search, no study was found that evaluated the protective effect of telmisartan and candesartan in isolated pylorus ligation induced ulcers. Both drugs in our study showed significant antisecretory and gastroprotective effects even in isolated pylorus ligation induced ulcer method. However, this needs to be confirmed by further studies.

The probable mechanism by which angiotensin receptor antagonists attenuate free and total acidity in pylorus ligation method could be attributed to their increased stimulation of gastrointestinal $HCO3^-$ secretion by a common pathway involving NO, PGs and bradykinin. This antisecretory activity could be because of increased NO generation by AT receptor antagonists [16] in response to enhanced acid secretion induced by vagally mediated mechanism, which occurs in pylorus ligation [15]. This NO reduces the gastric acid secretion under basal as well as stimulated conditions [16].

Both telmisartan and candesartan significantly reduced the ulcer index in indomethacin induced ulcer method. Telmisartan significantly reduced the gastric lesions as compared to candesartan ($p<0.05$) which suggests that telmisartan provided better gastroprotection than candesartan in indomethacin induced ulcers. The mechanisms by which indomethacin induces gastric ulceration are; depletion of prostaglandins, generation of reactive oxygen species, reduction in the mucosal blood flow and increased expression of cytokines like TNF-α which promotes neutrophil expression and in turn inflammation [17].

AT receptor blockers as stated earlier, enhance mucosal blood flow and prevent neutrophil infiltration by decreased expression of TNF- α and are therefore of benefit in indomethacin induced ulcer method.

In accordance with the present results, previous studies also suggested greater gastroprotective effect of telmisartan over candesartan in indomethacin induced ulcer method. In addition, this significant inhibitory effect of telmisartan on ulcer induction in previous studies was attributed to its PPAR-γ mediated action as telmisartan is reported to have the strongest PPAR-γ affinity among AT receptor blockers [18] which might be responsible for superior gastroprotection by virtue of additional antioxidant and anti-inflammatory actions.

We used ranitidine as a standard comparator, and found that though telmisartan and candesartan showed significant antiulcer activity in both ulcer models, it was less than ranitidine.

The results of this study in accordance with other reports from published literature suggest that both telmisartan and candesartan possess significant antiulcer activity with telmisartan being more gastroprotective than candesartan in indomethacin induced ulcer method.

CONCLUSION: The present study provides evidence that AT-II AT_1 antagonists might play a role in protection against gastric ulcers and offers insight into the mechanisms of gastric ulcer formation. This concept may provide a new path to develop important therapies for effectively treating two concurrently occurring diseases i.e., hypertension and peptic ulcer, with a single drug. However, further studies are greatly needed in this direction.

ACKNOWLEDGEMENT: We are grateful to Dr. Mogarekar (Professor and Head) and Dr Jyotsna Jaju, Department of Biochemistry, S.R.T.R Medical College, Ambajogai for helping us carrying out the biochemical investigations. Thanks to Dr D.B. Jadhav (Incharge of Animal House) for his kind assistance while performing the study.

REFERENCES:

1. Chen Liu, James MC. The gastrointestinal tract. In: Robbins and Cotran, editors. Pathologic basis of disease,7th ed. Noida, India: Elsevier Publications; 2004. p. 797-875.
2. Smirnov IuV, Oslopov VN, Bilich IL, Mendelevich VD. The epidemiological aspects of combined arterial hypertension and peptic ulcer. Ter Arkh. 1990; 62:48-50.
3. Sonnenberg A. Concordant occurrence of gastric and hypertensive diseases. Gastroenterology.1988; 95(1):42-8.
4. Fändriks L. The renin-angiotensin system and the gastrointestinal mucosa. Acta Physiol (Oxf). 2011; 201:157-67.
5. Garg M, Angus PW, Burrell LM, Herath C, Gibson PR, Lubel JS. Review article: the pathophysiological roles of the renin-angiotensin system in the gastrointestinal tract. Aliment Pharmacol Ther. 2012; 35(4):414-28.
6. Shiotani A, Nishi R, Yamanaka Y, Murao T, Matsumoto H, Tarumi K et al. Renin-angiotensin system associated with risk of upper GI mucosal injury induced by low dose aspirin: renin angiotensin system genes' polymorphism. Dig Dis Sci 2011;56: 465-471.
7. Brzozowski T, Ptak-Belowska A, Kwiecien S, Krzysiek-Maczka G, Strzalka M, Drozdowicz D *et al*. Novel concept in the mechanism of injury and protection of gastric mucosa: role of renin-angiotensin system and active metabolites of angiotensin. Curr Med Chem. 2012; 19(1):55-62.
8. Pawlik M, Pajdo R, Kwiecien S, Ptak-Belowska A, Sliwowski Z, Mazurkiewicz-Janik M *et al*. Nitric oxide (NO)- releasing aspirin exhibits a potent esophagoprotection in experimental model of acute reflux esophagitis. Role of nitric oxide and proinflammatory cytokines. J Physiol Pharmacol. 2011; 62(1):75-86.
9. Morsy M, Ashour O, Amin E, Rofaeil R. Gastroprotective effects of telmisartan on experimentally-induced gastric ulcers in rats. Pharmazie. 2009; 64:590-4.
10. Parmar NS, Desai JK. A review of the current methodology for the evaluation of gastric and duodenal anti-ulcer agents. Indian J Pharmacol. 1993; 25:120-35.
11. Shay M, Komarov SA, Fels D, Meranze D, Gruenstein H, Siplet H. A simple method for the uniform production of gastric ulceration in the rat. Gastroenterology. 1945; 5:43-61.
12. Hawk PB, Oser BL. Summerson HW In: Practical Physiological Chemistry. 12th Ed. Churchill London: McGraw Hill book Co; 1947: 347.
13. Downloaded from: http://rsbweb.nih.gov/ij/ download.html
14. Merai AH, Asad M, Prasad VS. Effect of Centrally Administered Losartan on Gastric and Duodenal Ulcers in Rats. Pharmacology. 2009; 84:93-8.
15. Alumets J, Ekelund L, Hakanson R, Hedenbro J, Rehfeld JF, Sundler F, Vallgren S. Gastric acid response to pylorus ligation in rats: Is gastrin or histamine involved? J. Physiol.1982; 323: 145-56.
16. Szlachcic A, Krzysiek-Maczka G, Pajdo R, Targosz A, Magierowski M, Jasnos K et al. The impact of asymmetric dimethylarginine (ADMA), the endogenous nitric oxide (NO) synthase inhibitor to the pathogenesis of gastric mucosal damage. Curr Pharm Des. 2013; 19: 90-97.
17. Taye A, Adel H. Saad et al. Role of rosiglitazone as a gastroprotective agent against indomethacin-induced gastric mucosal injury in rats. Gastroenterology Research. 2009; 2(6):324-32.
18. Fortunato Scalera, Jens Martens-Lobenhoffer, Alicja Bukowska, Uwe Lendeckel, Michael Tager *et al*. Effect of Telmisartan on Nitric Oxide–Asymmetrical Dimethylarginine System: Role of Angiotensin II Type 1 Receptor and Peroxisome Proliferator Activated Receptor γ Signaling During Endothelial Aging Hypertension. 2008:51; 696-703.

How to cite this article:
Rathod SS, Motghare VM and Deshmukh VS: Study of antiulcer activity of angiotensin receptor antagonists in experimentally induced gastric ulcers in rats. *Int J Pharm Sci Res* 2014; 5(2): 502-07.doi: 10.13040/IJPSR.0975-8232.5(2).502-07