*Int. J. Chem. Sci.: 13(3), 2015, 1511-1521*
*ISSN 0972-768X*
*www.sadgurupublications.com*

# EFFECT OF SOME CATIONS AS COUNTER IONS ON ULCER HEALING ACTIVITY OF GLYCYRRHETINIC ACID ON MALE ALBINO RATS

**SHARAD VISHT[a,b*] and G. T. KULKARNI[c]**

[a]Department of Pharmaceutical Science, Jawaharlal Nehru Technological University, Kukatpally, HYDERABAD – 500085 (A.P.) INDIA
[b]Meerut Institute of Engineering and Technology, NH-58, Bypass Road, Baghpat Crossing, MEERUT – 250005 (U.P.) INDIA
[c]School of Pharmacy, ITM University, NH-75, Jhansi Road, GWALIOR – 474001 (M.P.) INDIA

## ABSTRACT

Ulcer is a disease, which is treated with various drug salts as medicines. The present study was performed to find the contribution of counter ions on ulcer healing activity. The albino rats were selected and divided into six groups comprising minimum of 6 rats and all groups received 0.05 mL of 30% acetic acid (necrotizing agent) into the gastric wall to induce peptic ulcer. All groups received respective treatments for seven consecutive days by oral route except control group. On 8$^{th}$ day all rats were sacrificed and lesions measured. The prepared salts of glycyrrhetinic acid with different counter cation showed the variation in pH. The ulcer healing was maximum in case of glycyrrhetinic acid ammonium and minimum in case of glycyrrhetinic acid calcium, which may be due to increased protease activity, which is more at less acidic/alkaline pH so it may be a reason for interfering in ulcer healing. The one way analysis of variance showed p value < 0.0001, considered extremely significant. Histological studies revealed that ulcer control group exhibited severe damage of gastric mucosa as compared to treatment groups.

**Key words**: Glycyrrhetinic acid, Ulcer, Healing, Counter ion.

## INTRODUCTION

An imbalance between pepsin, acid, *Helicobacter pylori* infection, non-steroidal anti-inflammatory drugs (aggressive mechanism) and gastric mucus secretion, bicarbonate ions and prostaglandins (defensive mechanism) results in gastro duodenal mucosa ulcers[1].

Traditionally, Liquorice has been used as an expectorant, demulcent and in ulcer. Its major active component is a Saponin known as glycyrrhizin or glycyrrhizic acid (2-14%),

*Author for correspondence; E-mail: sharadvisht@gmail.com; Mo.: +91-9719302784

which has a similar structure and activity as the adrenal steroids. Glycyrrhizin, a glycoside, is its chief constituent, which hydrolyses on acidic hydrolysis into Glycyrrhizinic acid (triterpenoid) and two sugar molecules. It contains more than 30 flavonoids and isoflavonoids, including liquiritin and its derivatives. Glycyrrhizin has a cortisone-like effect that raises prostaglandin levels locally, increasing mucous secretion and promoting proliferation of cells in the stomach, stimulates gastric mucus production, enhances the rate of incorporation of various sugars into gastric mucosal glycoprotein, promotes mucosal cell proliferation, inhibits mucosal cell exfoliation, inhibits prostaglandin degradation, increases the release of PGEs, reduces the formation of thromboxane $B_2$ and regulates DNA and protein synthesis in gastric mucosa. It does not inhibit acid secretion[2-10].

Carbenoxolone sodium is a semi synthetic derivative of glycyrrhetinic acid, which has a steroid-like structure and is used for the treatment of peptic, oesophageal and oral ulceration and inflammation. It reversibly inhibits the conversion of cortisol to the inactive metabolite cortisone by blocking 11 β-hydroxysteroid dehydrogenase (11 β-HSD). 11 β-HSD also reversibly catalyzes the conversion of 7-ketocholesterol to 7-beta-hydroxycholesterol. The purpose of present study was to find the effect of counter ions on ulcer healing activity using various salts of glycyrrhetinic acid, which were prepared by changing the counter ions[11].

Some research concluded that protease activity is dependent on pH and so pH affects healing. The purpose of current study was to determine effect of counter ion on ulcer healing activity of various salts of glycyrrhetinic acid prepared by replacing counter ions[12,13].

## EXPERIMENTAL

### Material

The dried stolons of Liquorice (*Glycyrrhiza glabra*) were purchased from the local market of Meerut, India and authenticated at the National Bureau of Plant Genetic Resources (NBPGR), New Delhi. All the chemicals used were of analytical grade.

### Methodology

### Extraction and characterization of glycyrrhetinic acid

Dried stolons of Liquorice (*Glycyrrhiza glabra*) were used for extraction of Glycyrrhetinic acid (GA). Accurately weighed 100 g Liquorice powder was soaked in 500 mL pre-acidified distilled water for the hydrolysis of ether bond of glycyrrhizin resulting in one molecule of aglycon i.e., glycyrrhetinic acid and two molecules of glycon, i.e., glucuronic acid. The strong ammonia solution was added to the mixture and then

de-pigmentation was achieved by adding charcoal. The mixture was filtered crystallized and purified by column chromatography using chloroform: methanol (1:1) as mobile phase[14,15].

**Conversion of glycyrrhetinic ammonium into different salts**

Aqueous solution of glycyrrhetinic acid ammonium was treated with acid to convert ammonium salt of glycyrrhetinic acid into its base form. Then aqueous solution of NaOH, KOH, $Ca(OH)_2$ was added to prepare salt of respective alkali. Purification of prepared salt was done by column chromatography using chloroform: methanol (1:1) as mobile phase. Prepared salts were analysed by physical characterization, organoleptic properties, melting point, UV, IR, LOD and pH[14].

**Physical characterization**

Physical characterization was performed to confirm the physical state of salts like crystalline, amorphous state[14].

**Organoleptic properties**

Organoleptic properties include color, odor and taste[14].

**Melting point**

The measurement of the melting point is of major concern to identify the compound, which also reflects the solubility characteristics and purity of component. The melting point of glycyrrhetinic acid ammonium was determined by the capillary melting technique. Firstly, the melting point apparatus was calibrated using L-ascorbic acid AR and sodium carbonate AR. Then the small quantity of glycyrrhetinic acid ammonium was taken in a capillary tube and put in the digital melting apparatus, and average melting point glycyrrhetinic acid was determined[14].

**Loss on drying**

Accurately weighed 10 g of compound was placed in hot air oven, pre-adjusted at 100°C. Weigh the sample after each 1 hr until two constant weights are obtained[14].

**Determination of pH**

1% aqueous solution of compound in distilled water was prepared and the pH was checked with a standardized glass electrode[14].

**Determination of $\lambda_{max}$ by UV spectrophotometric analysis**

A stock solution of 1 mg/mL was prepared by weighing 100 mg of glycyrrhetinic

acid ammonium in 0.1 N HCl in 100 mL volumetric flask. Finally volume is made up to 100 mL. The 0.1 N HCl was used as blank/reference. Sample was scanned to determine the $\lambda_{max}$ with the help of Ultraviolet spectrophotometometer (Shimadzu 1700S). The dilutions were also scanned at $\lambda_{max}$ to measure absorbance and to prepare calibration curve of glycyrrhetinic acid ammonium[14].

## Fourier transform infrared spectroscopy

The Fourier transform infrared spectroscopy of the product was performed on FTIR (FTIR 8400S, CE, Software Irresolution). The perfectly dried glycyrrhetinic acid ammonium (1 mg) was mixed with potassium bromide KBr powder (10 mg) in a mortar pestle. Prepared mixture was then compressed into fine disc by KBr press at pressure of 15,000 Psi. Prepared disc was placed on window of IR spectrometer to determine various bonds and group present. The Fourier transform infrared spectroscopy of the product was obtained at a frequency of 400.1299 MHz, which showed a considerable difference in bands as shown in Figs. 2[14].

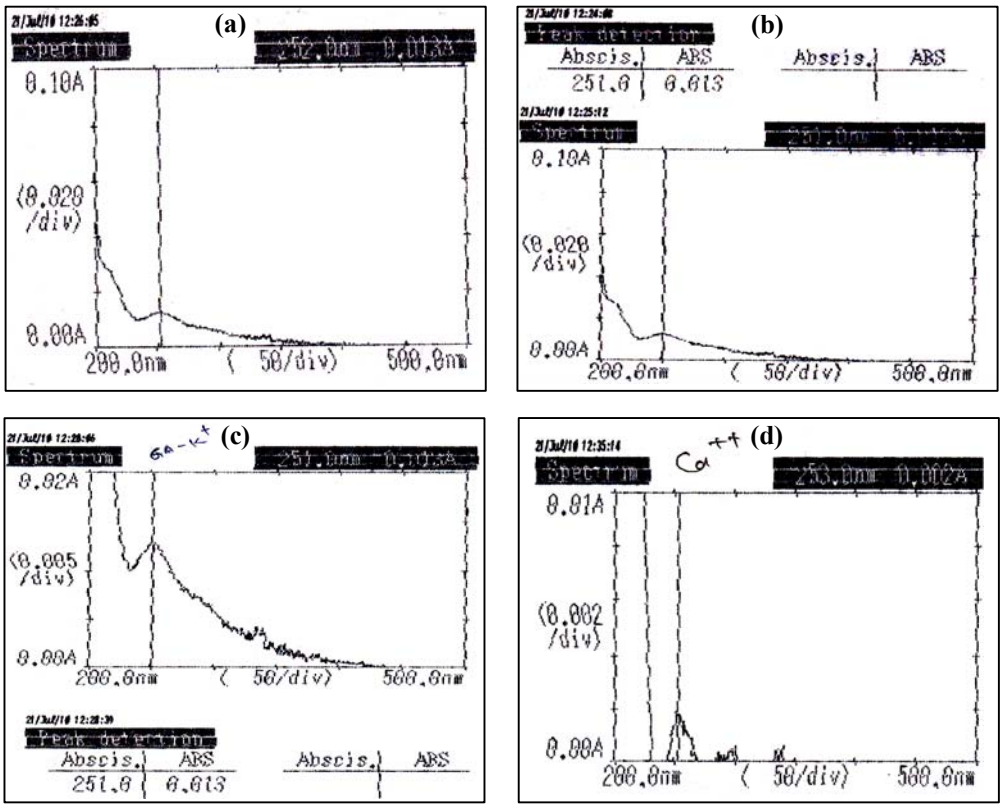


**Fig. 1: UV of Prepared salts of (a) Glycyrrhetinic acid ammonium, (b) Glycyrrhetinic acid sodium, (c) Glycyrrhetinic acid potassium, (d) Glycyrrhetinic acid calcium**

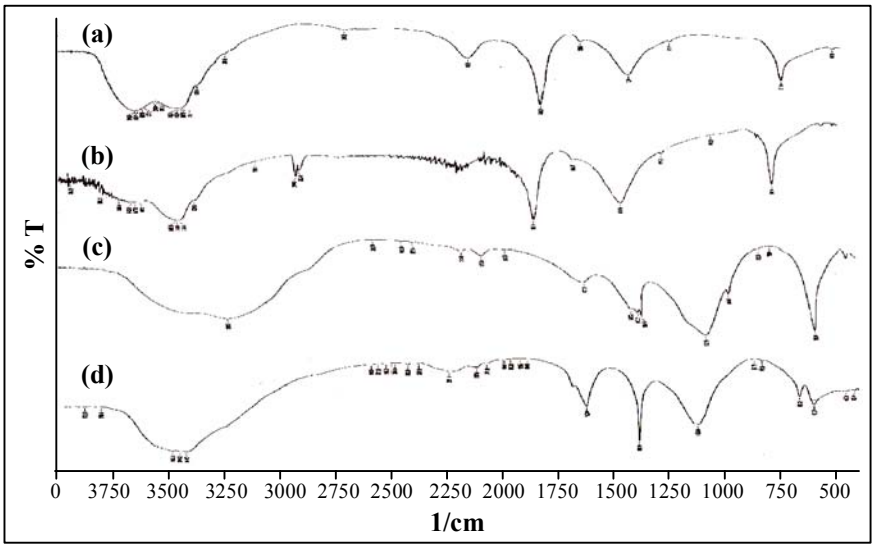


**Fig. 2: Fourier transform infrared spectroscopy of (a) Glycyrrhetinic acid ammonium, (b) Glycyrrhetinic acid sodium, (c) Glycyrrhetinic acid potassium, (d) Glycyrrhetinic acid calcium**

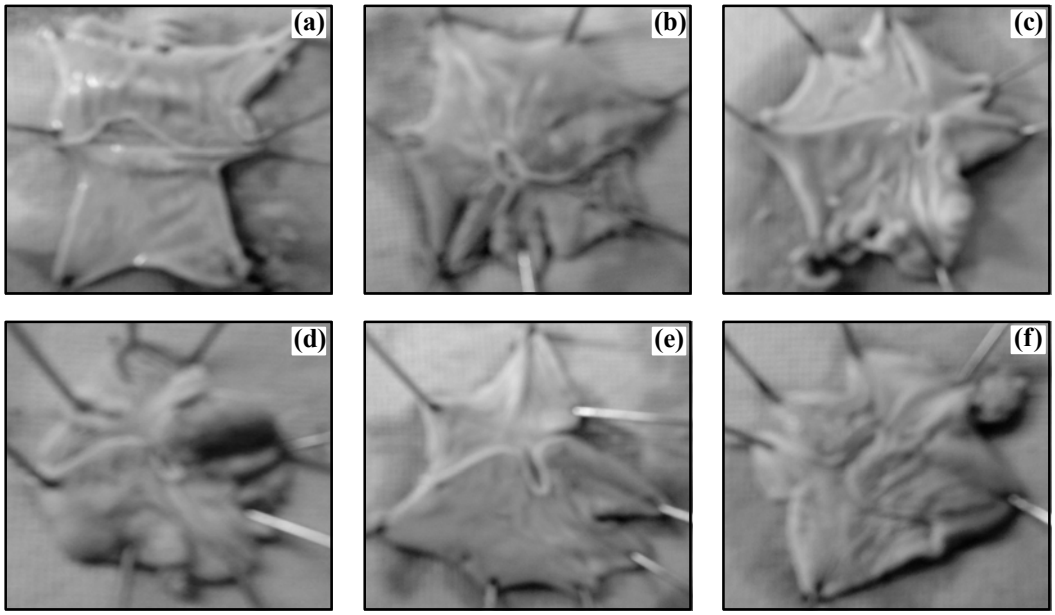


**Fig. 3: Ulceration in rat stomach in different groups; (a) Control, (b) Carbenoxolone sodium, (c) Glycyrrhetinic acid ammonium, (d) Glycyrrhetinic acid sodium, (e) Glycyrrhetinic acid potassium, (f) Glycyrrhetinic acid calcium**

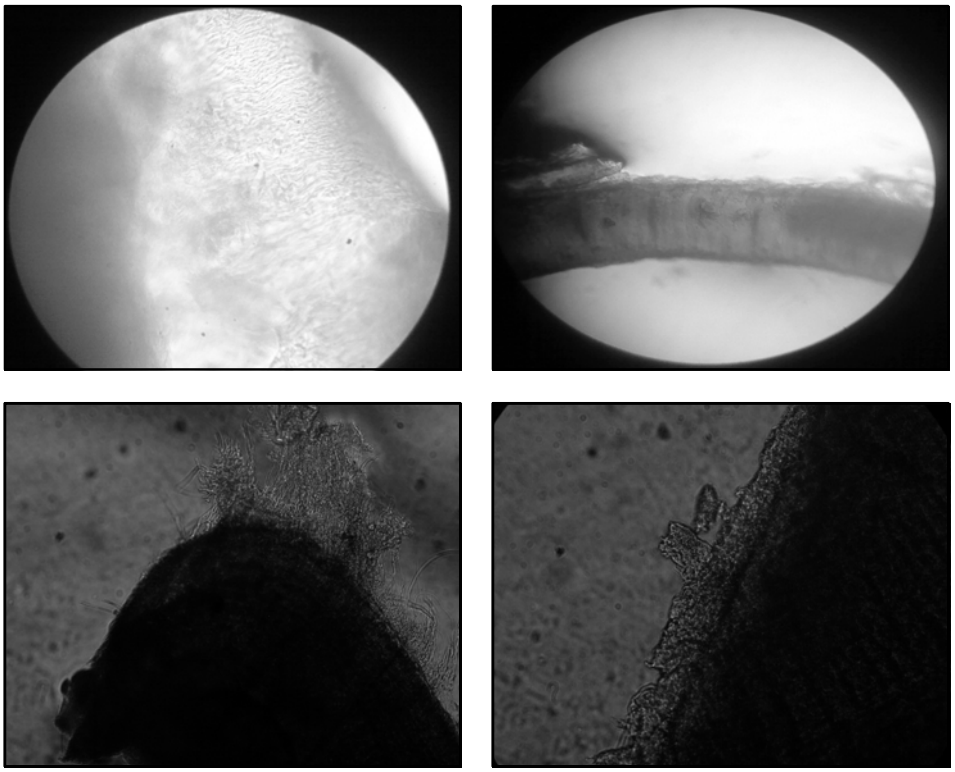

**Fig. 4: The histopathology showed the erosion of mucous layer on stomach wall**

### *In vivo* antiulcer activity

### Animal used

*In vivo* antiulcer study to ascertain the efficacy of salts was carried out in male Wistar albino rats weighing around 200 g. The animal experimental protocol was approved by the Institutional Animal Ethical Committee (No. 711/02/a/CPCSEA), India. The animals were housed in polypropylene cages and maintained at 24°C ± 2°C under a 12 hr light/dark cycle and were fed ad libitum with standard pellet diet and had free access to water[14].

### Administration and dosage

The 0.05 mL of 30% acetic acid was used as necrotizing agent, which was injected into the gastric wall of rat to induce peptic ulcer. Group A was considered as control. Group B, Group C, Group D, Group E, Group F received prescribed amounts of pure carbenoxolone sodium, glycyrrhetinic acid ammonium, glycyrrhetinic acid sodium,

glycyrrhetinic acid potassium and glycyrrhetinic acid calcium, respectively (Table 3). All doses were administered orally for seven consecutive days with normal diet.

**Acetic acid ulcer model**

Male albino rats of both sexes were selected and divided with six groups comprising minimum of 6 rats. All the animals were housed in standard cages. Ulcer was produced by using 0.05 mL of 30% acetic acid (necrotizing agent) into the gastric wall of rat to induce peptic ulcer. Standard and test drugs were administered orally for seven consecutive days. All animals were kept for one week and maintained under uniform diet, in specially constructed cages to prevent coprophagia during and after the experiment. At the end of the treatment ($8^{th}$ day), rats were fasted for 24 hrs, then anesthetized under ether atmosphere and sacrificed[16-27].

The percentage of ulcer protection was determined as follows:

$$\% \text{ Protective} = \frac{(\text{Control mean ulcer index} - \text{Test mean ulcer index})}{\text{Control mean ulcer index}} \times 100$$

**Histopathology**

Histopathology was performed to confirm the ulceration. The stomach tissue samples were fixed in phosphate buffered formalin for fixation of tissues. The tissue was dehydrated using ethyl alcohol and placed in paraffin/wax blocks. About 5 μm thick sections were cut using a rotary microtome. These sections were stained with hematoxylin using routine procedures. The slides were examined microscopically to determine patho-morphological changes like erosion of mucus layer[16-27].

**Table 1: Ulcer score**

| Status | Score |
|---|---|
| Normal stomach | 0 |
| Red coloration | 0.5 |
| Spot ulcer | 1.0 |
| Hemorrhazic streak | 1.5 |
| Ulcer | 2 |
| Perforation | 3 |

**Table 2: Analysis report**

| Properties | Ammonium | Sodium | Potassium | Calcium |
|---|---|---|---|---|
| Physical state | Solid crystalline | Solid crystalline | Solid crystalline | Solid crystalline |
| Organoleptic properties | White, odorless, characteristic taste | White, odorless, characteristic taste | White, odorless, characteristic taste | White, odorless, characteristic taste |
| Melting point | 292 ± 0.25°C | 293 ± 0.23°C | 293 ± 0.13°C | 293 ± 0.42°C |
| Loss on drying | 0.1% | 0.1% | 0.1% | 0.1% |
| pH of 1% solution | 4.2 ± 0.1 | 4.3 ± 0.1 | 4.5 ± 0.2 | 4.6 ± 0.1 |
| Test for Counter ion | White cloud of $NH_4Cl$ | | | White insoluble precipitate |
| $\lambda_{max}$ | 252 | 251 | 251 | 253 |

**Table 3: The ulcer healing activity of prepared salts of glycyrrhetinic acid**

| Group | Treatment | Dose (mg/Kg) | Ulcer Area ($mm^2$) (Mean ± S.E.M.) | % Inhibition |
|---|---|---|---|---|
| A | Control | - | 19.53 ± 0.34 | - |
| B | Carbenoxolone sodium | 25 | 6.73 ± 0.73 | 65.54019 |
| C | Glycyrrhetinic acid ammonium | 25 | 7.88 ± 0.23 | 59.65182 |
| D | Glycyrrhetinic acid sodium | 25 | 8.24 ± 0.53 | 57.8085 |
| E | Glycyrrhetinic acid potassium | 25 | 9.11 ± 0.11 | 53.35381 |
| F | Glycyrrhetinic acid calcium | 25 | 10.67 ± 0.34 | 45.3661 |

### Statistical analysis

The GraphPadPrism software was used to analyze the data using one way analysis of variance (ANOVA), where P value was determined to ensure whether results are considered statistically significant or insignificant[16-27].

## RESULTS AND DISCUSSION

The elemental analysis of various salts of glycyrrhetinic acid showed presence of types of ions as contamination.

Ulcer score were 1.5, 0.5, 1.0, 1.5, 1.5 and 1.5 for group A, B C, D, E, F, respectively. *In vivo* ulcer healing activity carried out using Wistar male albino rats indicated that ulcer healing was decreased in following order: Carbenoxolone sodium > Glycyrrhetinic acid Ammonium > Glycyrrhetinic acid sodium > Glycyrrhetinic acid potassium > Glycyrrhetinic acid calcium. The ulcer healing activity of Carbenoxolone sodium was more than Glycyrrehtinic acid ammonium because Carbenoxolone sodium is ester and it has low solubility and increase the duration of drug release itself. The results of *in vivo* ulcer healing study are shown in Table 3. The one way analysis of variance (ANOVA) was performed using GraphPadPrism software, which showed p value < 0.0001, considered extremely significant.

The study showed that the ulcer healing activity is affected by counter ions. The pH of 1% aqueous solutions of glycyrrhetinic acid salts was 4.2 ± 0.1, 4.3 ± 0.1, 4.5 ± 0.2 and 4.6 ± 0.1 due to different counter ion (ammonium, sodium, potassium and calcium).

As protease activity is more at alkaline pH (less acidic) so it may be a reason for interfering in ulcer healing. The ammonium salt will cause very less disturbances in gastric micro-environmental pH around ulcer cell while calcium salt cause more change in gastric micro-environmental pH. So the ulcer healing activity of ammonium salt of glycyrrhetinic acid was found to be maximum among prepared salts and minimum with calcium salt of glycyrrhetinic acid. The carbenoxolone sodium has better healing than ammonium salt of glycyrrhetinic acid because it is an ester, which has low aqueous solubility.

Several studies showed that acidic environment helps in wound healing by controlling wound infections, increasing antimicrobial activity, altering protease activity, releasing oxygen, reducing toxicity of bacterial end products, and enhancing epithelization and angiogenesis.

## CONCLUSION

It was concluded from study that ulcer healing depend on type of counter ion. The ulcer healing activity of ammonium salt of glycyrrhetinic acid was found to be maximum among prepared salts and minimum with calcium salt of glycyrrhetinic acid may be due to low protease activity at less acidic pH.

## REFERENCES

1. K. D. Tripathi, Essentials of Medical Pharmacology, Sixth Edition, Jaypee Brothers Medical Publishers (P) Ltd., India (2008) p. 635.

2. M. Ebadi, Pharmacodynamic Basis of Herbal Medicine, 2nd Ed. (2004) p. 597.

3. R Kataria, Hemraj, G. Singh, A. Gupta, S. Jalhan and A. Jindal, Asian J. Pharmaceut. Clin. Res., **6(1)**, 5-7 (2013).

4. T. L. Srinivas, S. M. Lakshmi, S. N. Shama, G. K. Reddy and K. R. Prasanna, J. Pharmacognosy Phytochem., **2(4)**, 91-97 (2013).

5. D. Kaur, A. C. Rana, N. Sharma and S. Kumar, J. Appl. Pharmaceut. Sci., **2(3)**, 160-165 (2012).

6. I. Kitagawa, Pure Appl. Chem., **74(7)**, 1189-1198 (2002).

7. A. Kumar and J. Dora, J. Pharamceut. Sci. Innovation, **2**, 1-4 (2014).

8. F. Borrelli and A. A. Izzo, Review Article: Phytotherapy Research, Phytother. Res., **14**, 581-591 (2000).

9. T. Lakshmi and R. V. Geetha, Int. J. Pharm. Pharmaceut. Sci., **3(4)**, 20-25 (2011).

10. S. Vispute and A. Khopade, J. Pharma BioSci., **2(3)**, 42-51 (2011).

11. http://gut.bmj.com/ on April 3, 2015, Published by group.bmj.com, Gut, **13**, 816-824 (1972).

12. G. Gethin, Wounds UK, **3(3)**, 52-56 (2007).

13. D. Armstrong, Aliment Pharmacol. Ther., **20** (Suppl. 5), 19-26 (2004).

14. S. Visht and G. T. Kulkarni, Int. J. Pharma Professional's Res., **5(1)**, 958-962 (2014).

15. S. H. Pawar, R. V. Shete, B. M. Patil, V. S. Pattankude, K. V. Otari and K. J. Kore, Int. J. Pharm. Life Sci., **1(8)**, 479-492 (2010).

16. D. Carbajal, V. Molina, M. Noa, Y. Ravelo, R. Mas and M. Valle, Int. J. Pharm. Pharmaceut. Sci., **5(4)**, 91-95 (2013).

17. T. Sato, H. Amano, Y. Ito, K. Eshima, T. Minamino, T. Ae, C. Katada, T. Ohno, K. Hosono, T. Suzuki, M. Shibuya, W. Koizumi and M. Majima, K. M. J., **43**, 57-66 (2013).

18. H. Soni, A. Shah, A. Paul and G. Patel, Int. J. Pharmacol. Clin. Sci., **3(3)**, 61-67 (2014).

19. M. Dorababu, T. Prabha, S. Priyambada, V. K. Agrawal, N. C. Aryya and R. K. Goel, Indian J. Experimental Biol., **42**, 389-397 (2004).

20. X. Xu, B. Xie, S. Pan, L. Liu, Y. Wang and M. D. C. Chen, Asia Pac. J. Clin. Nutr., **16** (Suppl 1), 234-238 (2007).

21. K. Amagase, M. Yokota, Y. Tsukimi and S. Okabe, J. Physiol. Pharmacol., **54(3)**, 349-360 (2003).

22. M. A. Abdulla, F. H. AL-Bayaty, L. T. Younis and M. I. Abu Hassan, J. Medicinal Plants Res., **4(13)**, 1253-1259 (2010).

23. S. K. Borra, R. K. Lagisetty and G. R. Mallela, African J. Pharm. Pharmacol., **5(16)**, 1867-1871 (2011).

24. M. Umamaheswari, K. Asokkumar, R. Rathidevi, A. T. Sivashanmugam, V. Subhadradevi and T. K. Ravi, J. Ethnopharmacol., **110**, 464-470 (2007).

25. V. C. Devaraj, Mohammed Asad and Satya Prasad, **45(4)**, 332-338 (2007).

26. V. Panda and M. Sonkamble, **2(3)**, 48-61 (2012).

27. S. Sultana, M. Akram, H. M. Asif and N. Akhtar, Int. J. Pharm., **5(5)**, 353-359 (2014).

*Revised : 11.06.2015* *Accepted : 13.06.2015*