ORIGINAL ARTICLE

# Effect of solubility enhancement and use of honey on anti-inflammatory and antibacterial activity of etodolac

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## Abstract

**Aim:** The aim of this study was to check the effect of solubility enhancement and use of honey on anti-inflammatory and antibacterial activity of etodolac (ETO). **Materials and Methods:** Ternary system of ETO was prepared using polyvinylpyrrolidone K30 (PVP K30) and hydroxypropyl β-cyclodextrin (HPB) by spray drying method. Design of experiment was used for preparation and optimization of ternary system. Formulations were prepared with and without addition of honey and were evaluated for its anti-inflammatory and antibacterial activity. Anti-inflammatory activity was evaluated using human red blood cell (HRBC) membrane stabilization and protein denaturation methods. Disc diffusion and well diffusion methods were used to determine antibacterial activity of prepared formulations. **Results and Discussion:** In HRBC membrane stabilization and protein denaturation method, the prepared ternary system formulations (without honey) achieved up to 83.3% and 83.4% inhibition; whereas, the pure sample of ETO had 58.1% and 57.8% inhibition, respectively. The antimicrobial activity was significantly affected by the type of bacteria used. In well diffusion method, run 8 showed the highest inhibitory activity of 35 ± 1.3 mm and 25 ± 1.2 mm (with honey) and 30 ± 1.1 mm and 18 ± 1.0 mm (without honey) against *Bacillus subtilis* and *Escherichia coli,* respectively. Addition of honey played a significant role in increasing the anti-inflammatory as well as antibacterial activity of all formulations. **Conclusion:** Use of PVP K30 and HPB in combination with honey was an effective approach to enhance the anti-inflammatory and antibacterial activity of ETO.

**Key words:** Design of experiments, etodolac, honey, hydroxypropyl β-cyclodextrin, polyvinylpyrrolidone K30, spray drying

## INTRODUCTION

Many chronic diseases such as rheumatoid arthritis, cardiovascular diseases and some types of cancer can be correlated with inflammatory response.[1] Penetration of an infectious agent, an antigen, or cell damage results in an inflammatory reaction. Most frequent sign of disease is inflammation. It is a fundamental biological process involving complex pathways that are often induced by the products of bacterial degradation from various microorganisms or even the body's own cells on damage and death.[2] Inflammation is the basic way in which the body reacts to irritation, infection, or other tissue injuries. The key features being a pain, redness, and swelling.[3] Extensive use of nonsteroids in the treatment of rheumatoid arthritis has evoked much interest for new drug development.[4]

Etodolac (ETO) is a novel nonsteroidal anti-inflammatory drug (NSAID) having anti-inflammatory, analgesic and antipyretic properties. It is used for the acute and chronic treatment of osteoarthritis and rheumatoid arthritis.[5,6] ETO has also found application in the treatment of ankylosing spondylitis, post-operative pain (dental, obstetric, or orthopedic surgery), and non-surgical pain (lower back pain, tendonitis, sports injury, or gout).[7] NSAIDs provide analgesic properties at lower doses, whereas, higher doses are required to produce anti-inflammatory action.[8] Its therapeutic effects

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are due to its ability to inhibit prostaglandin (PG) synthesis.[9] Due to its low solubility and high permeability ETO belongs to class II drug of biopharmaceutical classification system.[10]

Cyclodextrins (CDs) are cyclic oligosaccharides consisting of six (a CD), seven (b CD), eight (g CD), or more glucopyranose units linked by a-(1, 4) bonds.[11] CDs can interact with drug molecules to form inclusion complexes, major changes in drug properties such as enhanced solubility, chemical stability, physical profile, and pharmacological bioavailability have been reported.[12-14] Therefore, CDs are widely used in the preparation of various pharmaceutical products. Clinical studies showed greater efficacy of NSAIDs complexed with CDs in a variety of conditions. The preclinical studies demonstrated that the CD complexions were capable of producing an analgesic effect in several animal models, showing better effects than the drug alone. These preclinical studies also showed greater bioavailability of the drug with reduced side effects.[15]

Since ancient times, honey was valued as a flavorful sweetener. It also has an extensive history of traditional medicinal use. Studies have shown that honey has potential therapeutic value in the treatment of heart disease, cancer, cataracts, and several inflammatory diseases.[16] Honey also possesses antioxidant, antimicrobial, wound healing, and anti-inflammatory activities.[17] It is reported that honey contains at least 181 substances. Essentially, it is composed of a mixture of sugars, wax, enzymes, lipid, and other minor substances.[18,19] Honey is a rich source of flavonoids, phenolic acids, and other phytochemicals which give a wide range of biological effects.

In this context, this study aimed to evaluate the anti-inflammatory and antibacterial activity of ETO complex with CDs in the presence of honey through a systematic design of experiment (DoE) approach.

## MATERIALS AND METHODS

### Materials

ETO, polyvinylpyrrolidone K30 (PVP K30) were obtained from Lupin Research Park, Pune, India. Hydroxypropyl β cyclodextrin (HPB) was obtained from Roquette Pharma, France. Sodium chloride, Disodium hydrogen phosphate, and Potassium dihydrogen phosphate were procured from Oswal Scientifics, Pune, India. Kashmir honey was procured from Central Bee Research and Training Institute, Pune. Honey was stored at room temperature in airtight containers. All other reagents and chemicals used were of analytical grade.

### Cultures of Pathogenic Bacteria

The pathogenic bacteria *Escherichia coli* and *Bacillus subtilis* used in this study were obtained from National Chemical Laboratory, Pune. The bacteria were grown on nutrient agar (NA) (Hi media, India) and nutrient broth (Hi media, India) at 37°C for 24 h.

### Methods

#### *DoE*

Design Expert V10 software was used for designing of experiments. A CCD with α = 1 was employed as per standard protocol.[20,21] Experimental factors were studied at three levels each. The amount of PVP K30 (A) and HPB (B) was selected as experimental factors. All other processing variables and formulation ingredients were kept constant throughout the study. The central point (0,0) was studied in quintuplicate. Design expert software gave 19 experimental runs with different combinations of factors as depicted in Table 1. Percent human red blood cell (HRBC) membrane stabilization, inhibition of protein denaturation, diameter of the zone of inhibition by disc diffusion and well diffusion methods were taken as response variables.

### Preparation of Spray Dried Ternary System

Appropriate weight of ETO was added in 99.8% methanol. Required amounts of PVP K30 and HPB (as per DoE) were

**Table 1:** Central composite design

| Run | ETO mg | PVP K30 (A) mg | HPB (B) mg | Honey (C) mg |
|---|---|---|---|---|
| 1 | 100 | 150 | 150 | 80 |
| 2 | 100 | 50 | 150 | 120 |
| 3 | 100 | 100 | 100 | 133.6359 |
| 4 | 100 | 150 | 50 | 120 |
| 5 | 100 | 100 | 100 | 100 |
| 6 | 100 | 15.91036 | 100 | 100 |
| 7 | 100 | 150 | 50 | 80 |
| 8 | 100 | 150 | 150 | 120 |
| 9 | 100 | 100 | 100 | 100 |
| 10 | 100 | 100 | 100 | 66.36414 |
| 11 | 100 | 50 | 50 | 120 |
| 12 | 100 | 50 | 150 | 80 |
| 13 | 100 | 100 | 184.0896 | 100 |
| 14 | 100 | 50 | 50 | 80 |
| 15 | 100 | 100 | 100 | 100 |
| 16 | 100 | 100 | 100 | 100 |
| 17 | 100 | 184.0896 | 100 | 100 |
| 18 | 100 | 100 | 15.91036 | 100 |
| 19 | 100 | 100 | 100 | 100 |
| 20 | 100 | - | - | - |

ETO: Etodolac, PVP: Polyvinylpyrrolidone, HPB: Hydroxypropyl β-cyclodextrin

added in purified water [Table 1]. The solutions were further mixed and were kept for sonication for 20 min and spray dried (Labultima LU-222). The drying conditions were as follows: Inlet temperature 90°C; outlet temperature 70°C; aspirator 65%; feed rate 12%; atomization air pressure 1.75 kg/cm$^2$.

## Anti-inflammatory Activity

### *HRBC membrane stabilization method*

#### *Collection of human erythrocyte suspension*

The blood sample was collected from a healthy volunteer who had not taken any NSAIDs for 2 weeks before the experiment and collected in heparinized vacutainer. The collected blood was mixed with equal volume of 2% dextrose, 0.8% sodium citrate, 0.05% citric acid, and 0.42% sodium chloride in water. The blood was centrifuged at 3000 rpm, and packed cells were washed with isosaline (0.85%, pH 7.2) and 10% v/v suspension was made with isosaline.

#### *Hypotonic solution-induced hemolysis*

The anti-inflammatory activity of ETO and its ternary system in presence and absence of honey was determined by HRBC membrane stabilization method. Appropriate dilutions were made of drug and carrier formulations (as mentioned in Table 1) with and without honey to get 150 µg/ml concentration of the drug. The assay mixture contained 1 ml phosphate buffer (0.15 M, pH 7.4), 2 ml of hyposaline (0.36%), 0.5 ml of HRBC suspension, and 1 ml of 150 µg/ml drug from various formulations. Drugs were omitted in the blood control, while drug control did not contain the erythrocyte suspension. All the assay mixtures were kept at 37 ± 2°C for 30 min and centrifuged. The hemoglobin content in the supernatant solution was estimated at 560 nm.[22] The percentage of HRBC membrane stabilization or protection was calculated using the following formula.

$$\text{Percent protection} = \frac{(1 - \text{ODt})}{\text{ODc}} \times 100$$

Where,
ODt = absorbance of test sample,
ODc = absorbance of control.

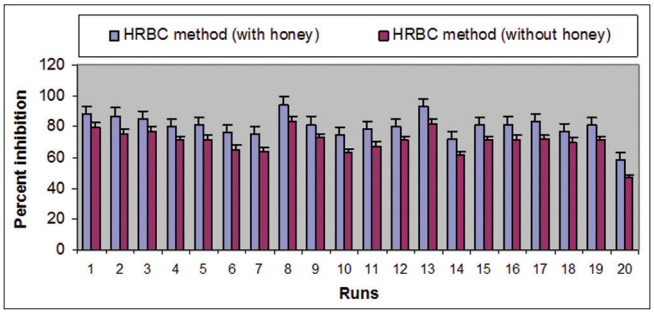


**Figure 1:** Effect of solubility enhancement and use of honey on anti-inflammatory activity of etodolac by human red blood cell membrane stabilization method (mean ±SD; *n*=3)

### *Protein denaturation method*

The reaction mixture (5 ml) consisted of 0.2 ml of egg albumin (from fresh hen's egg), 2.8 ml of phosphate buffered saline (PBS, pH 6.4) and 2 ml of drug and carrier formulations (as mentioned in Table 1) of 150 µg/ml concentration with and without addition of honey. A similar volume of double-distilled water served as control. Then, the mixtures were incubated at (37±2°C) in a BOD incubator (Labline Technologies) for 15 min and then heated at 70°C for 5 min. After cooling, absorbance was measured at 660 nm (SHIMADZU, UV 1800). Pure ETO was used as reference drug and treated similarly for determination of absorbance.[23] The percentage inhibition of protein denaturation was calculated using the following formula:

$$\% \text{ inhibition} = 100 \times (\text{Vt}/\text{Vc} - 1)$$

Where,
Vt = absorbance of test sample,
Vc = absorbance of control.

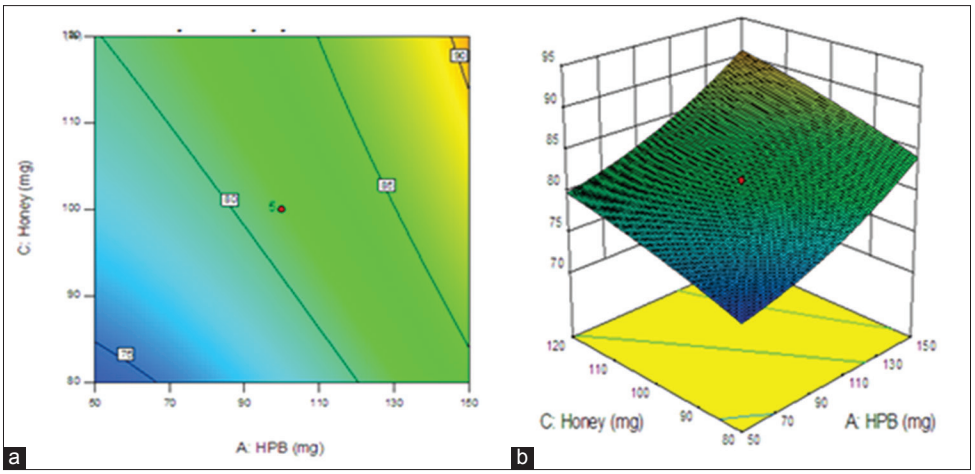


**Figure 2:** Effect of honey and hydroxypropyl β-cyclodextrin on anti-inflammatory activity of etodolac (percent inhibition) by human red blood cell membrane stabilization method, (a) contour graph, (b) 3D graph

### Antibacterial activity

#### *Antibacterial activity using disc diffusion method*

Antibacterial activity of various formulations was determined by disc diffusion method on NA agar (Hi media, India). The pathogenic bacteria cultures were swabbed on NA plates. Discs were overlaid overnight with drug and carrier formulations (as mentioned in Table 1) of 5 mg/ml concentration with and without addition of honey and then dried at 45°C for 24 h. Discs were placed on swabbed agars and incubated at 37 ± 2°C for 24 h. Diameter of the zone of inhibition was carefully measured, and the results were expressed in millimeter.[24] This experiment was done in triplicate and mean with standard deviation was calculated.

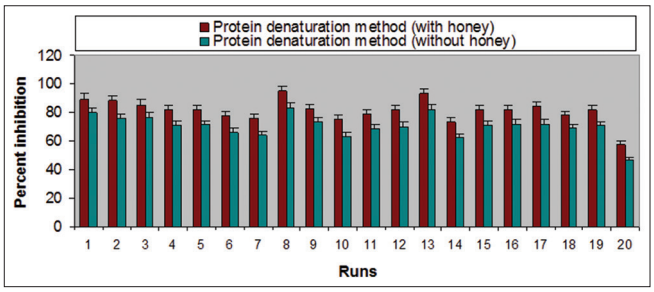


**Figure 3:** Effect of solubility enhancement and use of honey on anti-inflammatory activity of etodolac by protein denaturation method (mean±SD; *n*=3)

#### *Antibacterial activity using well diffusion method*

Antibacterial activity of ETO was determined by well diffusion method as described by Perez *et al*. with slight modifications.[25] Various samples of ETO were prepared using deionized water. Nutrient broth was used for preparation of culture of pathogenic bacteria (Hi media, India). 1% of pathogenic bacteria (109 CFU/ml) was added and mixed carefully in NA once its temperature reached 40°C. NA with 1% of pathogenic bacteria was poured to Petri plates. Sterile cork borer was used to prepare wells and left to dry at room temperature. After that, prepared ETO formulations (5 mg/ml) with and without addition of honey were poured to the wells individually and kept at 37 ± 2°C for 24 h. Inhibition zone diameter was carefully measured, and values were expressed as mean with standard deviation. The experiment was done in triplicate.

## RESULTS AND DISCUSSION

Use of animals in experimental pharmacological research has certain problems such as ethical issues and the lack

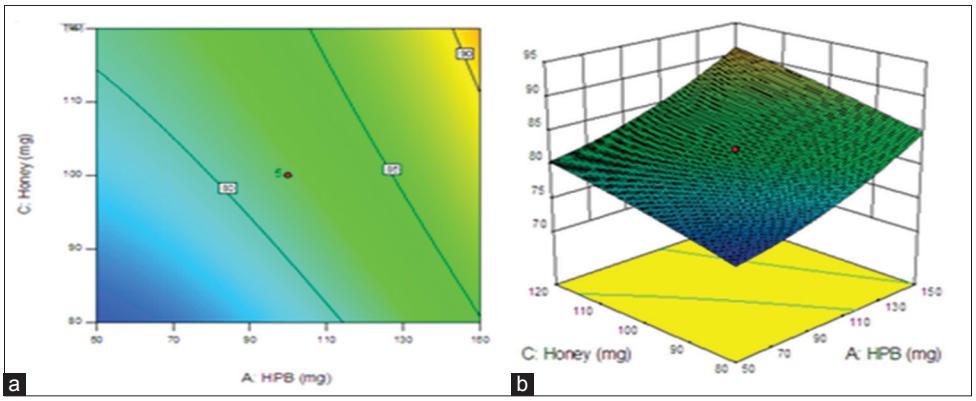


**Figure 4:** Effect of honey and hydroxypropyl β-cyclodextrin on anti-inflammatory activity of etodolac (percent inhibition) by protein denaturation method, (a) contour graph, (b) 3D graph

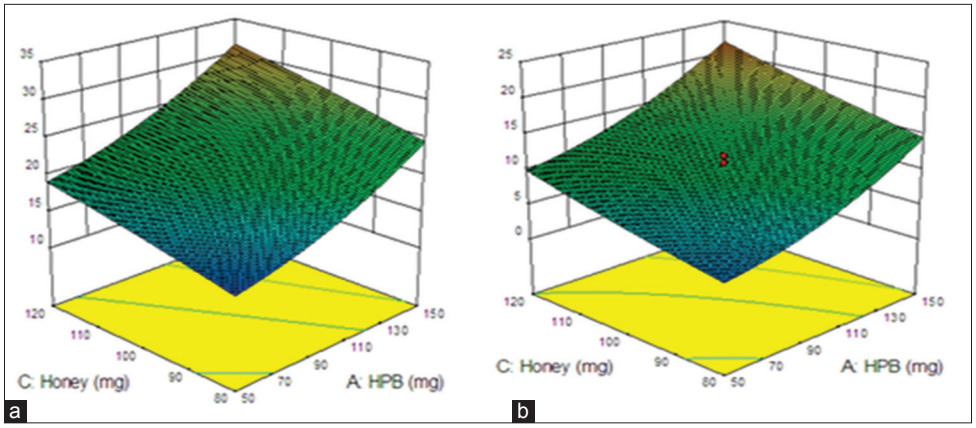


**Figure 5:** (a-b)Effect of honey and hydroxypropyl β-cyclodextrin on antibacterial activity of etodolac (zone of inhibition) by disc diffusion method, *Bacillus subtilis* (left side) and *Escherichia coli* (right side)

of rationale for their use when other suitable methods are available. Hence, in this study anti-inflammatory and antibacterial activities of ETO and its ternary system was carried out using *in-vitro* methods. Anti-inflammatory activity was evaluated using HRBC membrane stabilization and protein denaturation bioassay while antibacterial activity was evaluated by disc diffusion and well diffusion methods. The results were as follows.

## Anti-inflammatory activity

### *HRBC membrane stabilization method*

Anti-inflammatory activity of any agent can be correlated with its protective effect on heat and hypotonic saline-induced erythrocyte lyses. As human RBC membrane is analogs to the lysosome membrane, the detail idea about the inflammatory process can be obtained by studying the inhibition of RBC hemolysis.[22] All the 19 formulations prevented RBC hemolysis greater than that showed by plain ETO [Figure 1]. Run 8 (without honey) exhibited superior HRBC membrane stabilization (83.4%) greater than that of plain ETO (47.1%). In addition, it was also noticed that as the concentration of carriers in the formulation increased, membrane stabilization effect of the drug was increased [Figure 2]. This might be due to the improved solubilization of the drug by PVP K30 and HPB, which in turn improves the membrane stabilization potential of the drug. Honey exhibited its potency by preventing HRBC hemolysis in a hypotonic solution. All the formulations containing honey had shown prevention of HRBC hemolysis to the higher extent, compared to that shown by the formulations without honey. Formulations containing honey showed greater anti-inflammatory effect, may be due to the inhibition of cyclooxygenase-1 and cyclooxygenase-2, as Reyes-Gordillo *et al*. reported reductions in concentrations of PG such as PGE2, PGF2α (PGF2a), and thromboxane B2 in human plasma on ingestion of diluted honey.[26] Run 8 (with honey) exhibited 94.4% of RBC membrane protection [Figure 1].

### *Protein denaturation method*

One of the features of several NSAID is their ability to stabilize heat treated albumin (prevent denaturation) at the physiological pH (pH: 6.2-6.5).[13] One of the well-documented causes of inflammatory and arthritic disease was denaturation of tissue proteins. In certain arthritic diseases production of autoantigens may be due to denaturation of proteins *in vivo*.[10] For anti-inflammatory drug development, agents that can prevent protein denaturation would be worthwhile. This study revealed that all 19 formulations [Figure 3] were more effective in preventing protein denaturation as compared to plain ETO. Use of honey in the formulations again proved its role by improving percent inhibition of protein denaturation. Run 8 (without honey) exhibited 83.4% of protein denaturation prevention while run 8 (with honey) showed 94.8% protein denaturation prevention [Figure 3]. From Figure 4 it can be noted that as the concentration of HPB and honey in the formulations increased there was increase in anti-inflammatory activity of all formulations.

## Antibacterial activity

### *Antibacterial activity using disc diffusion method*

From Figure 5 it can be noted that concentration of HPB and honey was having a direct correlation with antibacterial activity of ETO. All the 19 formulations (with and without honey) showed variable inhibitory activities against the target bacteria by the disc diffusion method [Figures 6 and 7]. The inhibitory activity was significantly affected by the type of bacteria used. *B. subtilis* was greatly inhibited by all formulations. Run 8 (without honey) showed the highest inhibitory activity with an inhibitory zone of 32 ± 1.14 mm; while *E. coli* was inhibited to a lesser extent with an inhibitory zone of 19 ± 1.1 mm [Figure 6]. Plain ETO showed the inhibitory activity of 3.0±1.2 mm against *B. subtilis* while it did not inhibit *E. coli*. Addition of honey played a significant role in increasing the antibacterial activity of all formulations [Figure 7]. This may be due to the enzymatic production of hydrogen peroxide by honey, osmotic effect of its high sugar content and low moisture content along with its acidic properties of a gluconic acid.[14,18] Run 8 with honey showed the highest inhibitory activity of 35.00 ± 1.11 mm and 25.00 ± 1.24 mm against *B. subtilis* and *E. coli,* respectively.

### *Antibacterial activity using well diffusion method*

In well diffusion method, similar correlation of HPB and honey with respect to antibacterial activity of ETO was

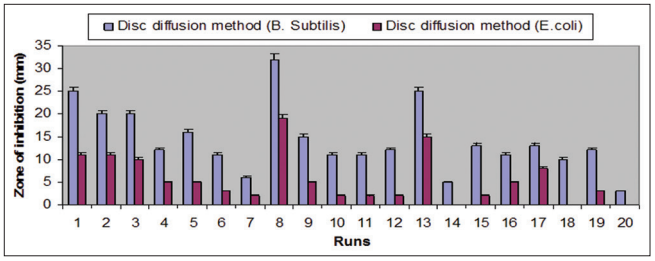


**Figure 6:** Effect of solubility enhancement on antibacterial activity of etodolac by disc diffusion method without using honey (mean±SD; *n*=3)

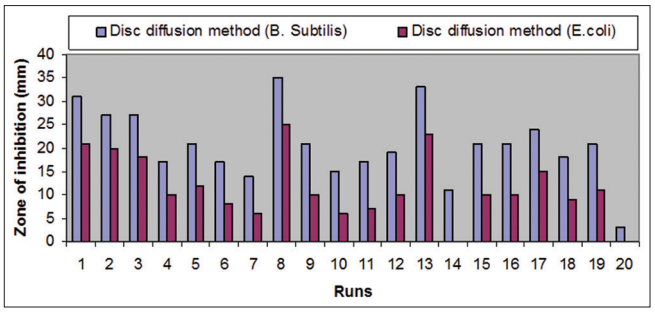


**Figure 7:** Effect of solubility enhancement on antibacterial activity of etodolac by disc diffusion method using honey (mean±SD; *n*=3)

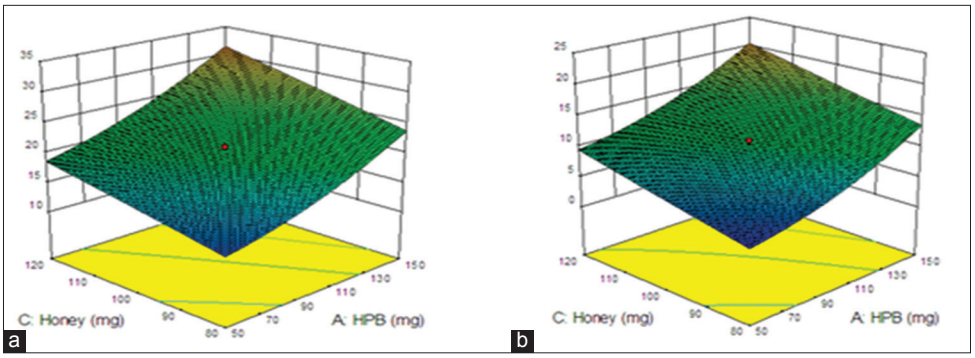


**Figure 8:** Effect of honey and hydroxypropyl β-cyclodextrin on antibacterial activity of etodolac (zone of inhibition) by well diffusion method, *Bacillus subtilis* (left side) and *Escherichia coli* (right side)

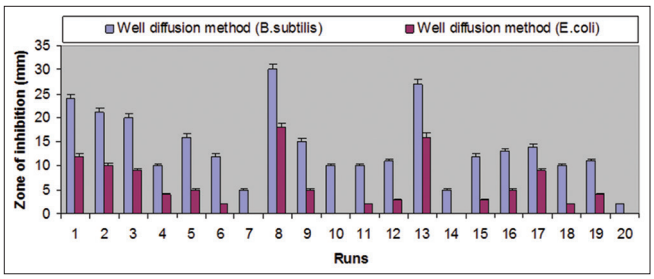


**Figure 9:** Effect of solubility enhancement on antibacterial activity of etodolac by well diffusion method without using honey (mean±SD; *n*=3)

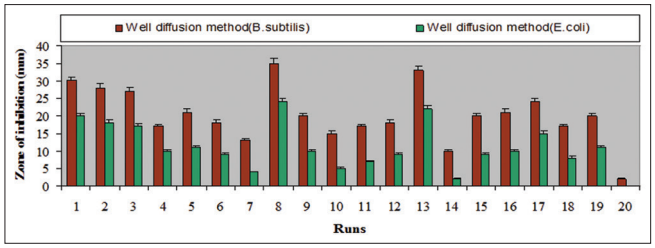


**Figure 10:** Effect of solubility enhancement on antibacterial activity of etodolac by well diffusion method using honey (mean±SD; *n*=3)

observed as that of disc diffusion method [Figure 8]. Addition of honey in all 19 runs improves the antibacterial effect of the formulations. The degree of inhibition was affected by the type of bacteria as well as by the type of formulation. The diameters of growth inhibitory zones for formulations without honey were ranging between 0 and 30 mm [Figure 9]. Among the bacteria evaluated, *B. subtilis* was easily inhibited by all the tested samples, while *E. coli* was the most difficult to inhibit. Microbial growth was significantly inhibited by all honey containing formulations with inhibitory zone diameter ranging between 0 and 35 mm [Figure 10]. Run 8 showed the highest inhibitory zone of 35 ± 1.3 mm and 25 ± 1.2 mm (with honey) and 30 ± 1.1 mm and 18 ± 1.0 mm (without honey) against B. *subtilis* and *E. coli,* respectively. Figure 8 showed the effect of honey on antibacterial activity of ETO. As the amount of honey in the formulation increased, there was a directly proportional increase in antibacterial activity. This proved the role of honey in combination with HPB and PVP K30 in increasing the antibacterial activity of ETO.

## CONCLUSION

Spray drying method was effectively applied to increase the solubility of ETO. In this study, ETO was complexed with HPB and PVP K30 which was subsequently formulated with and without honey. Complexation increases the solubility of ETO in gastric simulated fluid and increase availability of the drug for anti-inflammatory action. Results of HRBC membrane stabilization method, protein denaturation method, well diffusion method and disc diffusion method showed that all the formulations showed greater anti-inflammatory and antibacterial activity as compared to pure ETO. Addition of honey played a significant role in increasing the anti-inflammatory and antibacterial activity of all formulations. Hence from this study, it can be concluded that use of PVP K30 and HPB in combination with honey was an effective approach to enhance the anti-inflammatory and antibacterial activity of ETO.

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## REFERENCES

1. Laaboudi W, Ghanam J, Aissam H, Merzouki MA, Benlemlih M. Anti-inflammatory and analgesic activities of olive tree extract. Int J Pharm Pharm Sci 2016;7:414-9.
2. Souto AL, Tavares JF, da Silva MS, Diniz Mde F, de Athayde-Filho PF, Barbosa Filho JM.

Anti-inflammatory activity of alkaloids: An update from 2000 to 2010. Molecules 2011;16:8515-34.
3. Mbiri JW, Kasili S, Patrick K, Mbinda W, Piero NM. Anti-inflammatory properties of methanolic bark extracts of *Terminalia brownii* in wistar albino rats. Int J Curr Pharm Res 2016;3:100-4.
4. Shekhar C, Bahuguna YM, Singh V. Anti-inflammatory activity of ethanolic stem extracts of *Rubia cordifolia* linn. in rats. Int J Res Ayurveda Pharm 2010;1:126-30.
5. Sinha VR, Chadha AR, Goel H. Enhancing the dissolution of hydrophobic guests using solid state inclusion complexation: Characterization and *in vitro* evaluation. J Incl Phenom 2010;3:381-92.
6. Humber LG. Etodolac: The chemistry, pharmacology, metabolic disposition, and clinical profile of a novel anti-inflammatory pyranocarboxylic acid. Med Res Rev 1987;7:1-28.
7. Bellamy N. Etodolac in the management of pain: A clinical review of a multipurpose analgesic. Inflammopharmacology 1997;5:139-52.
8. Spencer-Green G. Low dose etodolac in rheumatoid arthritis: A review of early studies. J Rheumatol Suppl 1997;47:3-9.
9. Inoue N, Nogawa M, Ito S, Tajima K, Kume S, Kyoi T. The enantiomers of etodolac, a racemic anti-inflammatory agent, play different roles in efficacy and gastrointestinal safety. Biol Pharm Bull 2011;34:655-9.
10. Barakat NS. Etodolac-liquid-filled dispersion into hard gelatin capsules: An approach to improve dissolution and stability of etodolac formulation. Drug Dev Ind Pharm 2006;32:865-76.
11. Scarpignato C. Piroxicam-ß-cyclodextrin: A GI safer piroxicam. Curr Med Chem 2013;20:2415-37.
12. Quintans Jde S, Menezes PP, Santos MR, Bonjardim LR, Almeida JR, Gelain DP, *et al.* Improvement of p-cymene antinociceptive and anti-inflammatory effects by inclusion in ß-cyclodextrin. Phytomedicine 2013;20:436-40.
13. Tejashri G, Amrita B, Darshana J. Cyclodextrin based nanosponges for pharmaceutical use: A review. Acta Pharm 2013;63:335-58.
14. Pinho E, Grootveld M, Soares G, Henriques M. Cyclodextrins as encapsulation agents for plant bioactive compounds. Carbohydr Polym 2014;101:121-35.
15. Brito RG, Araújo AA, Quintans JS, Sluka KA, Quintans-Júnior LJ. Enhanced analgesic activity by cyclodextrins - a systematic review and meta-analysis. Expert Opin Drug Deliv 2015;12:1677-88.
16. Al-Mamary M, Al-Meeri A, AL-Habori M. Antioxidant activities and total phenolics of different types of honey. Nutr Res 2002:22:1041-7.
17. Basualdo C, Sgroy V, Finola MS, Marioli JM. Comparison of the antibacterial activity of honey from different provenance against bacteria usually isolated from skin wounds. Vet Microbiol 2007;124:375-81.
18. Gomes S, Dias LG, Moreira LL, Rodrigues P, Estevinho L. Physicochemical, microbiological and antimicrobial properties of commercial honeys from Portugal. Food Chem Toxicol 2009;48:544-48.
19. White JW Jr. Composition of Honey. In: Crane E, editor. Honey: A Comprehensive Survey. London: Heinemann; 1975. p. 180-94.
20. Singh B, Kumar R, Ahuja N. Optimizing drug delivery systems using systematic "design of experiments."Part I: Fundamental aspects. Crit Rev Ther Drug Carrier Syst 2004;22:27-105.
21. Singh B, Dahiya M, Saharan V, Ahuja N. Optimizing drug delivery systems using systematic "design of experiments." Part II: Retrospect and prospects. Crit Rev Ther Drug Carrier Syst 2005;22:215-94.
22. Ingale S, Dange H, Chendekar S. Evaluation of anti-inflammatory activity of some guggul containing polyherbal formulations. Invent Rapid Plant Activa 2015;3:1-3.
23. Chandra S, Chatterjee P, Dey P, Bhattacharya S. Evaluation of *in vitro* anti-inflammatory activity of coffee against the denaturation of protein. Asian Pac J Trop Biomed 2012;2:S178-80.
24. Bauer AW, Kirby WM, Sherris JC, Turck M. Antibiotic susceptibility testing by a standardized single disk method. Am J Clin Pathol 1966;45:493-6.
25. Perez C, Pauli M, Bazerque P. An antibacterial assay by agar well diffusion method. Acta Bio Med Exp 1990;15:113-5.
26. Reyes-Gordillo K, Segovia J, Shibayama M, Vergara P, Moreno MG, Muriel P. Curcumin protects against acute liver damage in the rat by inhibiting NF-kappa B, proinflammatory cytokines production and oxidative stress. Biochem Biophys Acta 2007;6:989-96.

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