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Research Article

# Evaluation of analgesic activity and acute toxicity of ketoprofen-nicotinamide multicomponent solids

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## ABSTRACT

The objective of this study was to evaluate the analgesic activity and acute toxicity of the ketoprofen-nicotinamide multicomponent solid. The preparation of multicomponent solids was carried out by the solvent evaporation method. The characterization of multicomponent solids was carried out by powder x-ray diffractometer (PXRD), differential scanning calorimeter (DSC), and fourier transform infrared spectrometer (FTIR). Solubility test was carried out by shaking method and evaluation of analgesic activity-toxicity was carried out *in vivo* in mice strain BALB/c. The results showed that the ketoprofen-nicotinamide multicomponent solid formed a new solid phase with different characteristics from the initial components. The ketoprofen-nicotinamide multicomponent solid showed a significantly ($p$<0.05) increased solubility, which was 1.3 times compared to the solubility of pure ketoprofen. In the evaluation of analgesic activity, the treatment group of multicomponent solid at a dose of 3.25 mg/kg body weight showed significantly increased pain inhibition ($p$<0.05) compared to the treatment group of pure ketoprofen. The toxicity evaluation in experimental animals showed that the multicomponent solids did not cause a significant ($p$>0.05) increase in SGOT and SGPT levels compared to the control group. Observations on the stomach histology of experimental animals showed that the ketoprofen-nicotinamide multicomponent solid gave a lighter infiltration of neutrophil inflammatory cells when compared to the pure ketoprofen group.

**Keywords:**
Acute toxicity, Analgesic activity, Ketoprofen, Multicomponent solids

## 1. INTRODUCTION

Ketoprofen is a class of non-steroidal anti-inflammatory drugs derived from propionic acid which have anti-inflammatory, analgesic and, antipyretic effects. It has activity through an inhibitory mechanism on the production of prostaglandins[1-2]. Based on the biopharmaceutical classification system, ketoprofen is included in class II compounds which have high permeability but low solubility[1]. The low solubility is known to affect the absorption rate and bioavailability of drugs in the blood[3].

One method that is known to be used to increase the solubility and dissolution rate of ketoprofen is through the formation of multicomponent solids. The formation of multicomponent solids of ketoprofen with nicotinamide showed solubility of 1.3 times higher than that of pure ketoprofen[4]. Thus, the multicomponent solid of ketoprofen has the opportunity to be formulated into a pharmaceutical dosage form with better absorption and bioavailability.

*In vitro* solubility data of a drug cannot fully predict the pharmacological activity of the drug in the body[5]. This is because the pharmacokinetic properties of a drug *in vivo* are not only determined by its solubility, but are also influenced by other physiological factors such as the transit time of the drug in the gastrointestinal tract and its stability in the luminal fluid[6]. Therefore, it is necessary to evaluate the pharmacological activity of a drug in animal experiments so that it can confirm its effect on living tissue. In this study, the analgesic activity and toxicity of ketoprofen multicomponent solids were evaluated *in vivo*

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in experimental animals. The preparation of ketoprofen multicomponent solids was carried out by solvent evaporation method using nicotinamide coformers. Multicomponent solids were characterized by powder x-ray diffraction (PXRD), differential scanning calorimeter (DSC), fourier transform infrared spectrometer (FTIR) and apparent solubility test. Evaluation of analgesic activity was carried out *in vivo* in experimental animals using the writhing test method and followed by an acute toxicity test.

## 2. MATERIALS AND METHODS

### 2.1. Materials

Ketoprofen was purchased from Tokyo Chemical Industry (Japan) and nicotinamide was purchased from Western Drugs (India). 2-propanol, carboxymethylcellulose sodium (CMC Na), acetic acid (glacial), and sodium chloride were obtained from Merck (Germany).

### 2.2. Preparation of ketoprofen-nicotinamide multicomponent solid

The preparation of ketoprofen multicomponent solids was carried out by the solvent evaporation method as mentioned in the previous study[4]. The mixture of ketoprofen and nicotinamide in a molar ratio of 2:1 was put in a beaker, and then 2-propanol was added and stirred using a magnetic stirrer for 30 minutes at 25°C. The glass beaker containing the ketoprofen-nicotinamide solution was then covered with aluminum foil which had been given small holes and then left at room temperature so that all the solvent evaporated and a dry solid was produced. The solids were crushed using a mortar and stamper and sieved through an 80 mesh sieve. The powder of ketoprofen-nicotinamide multicomponent solid was stored in a desiccator until further testing was carried out.

### 2.3. Characterization of ketoprofen-nicotinamide multicomponent solids

#### *2.3.1. Powder x-ray diffraction (PXRD)*

PXRD diffractograms were collected using an X-ray diffractometer with CuKα radiation (1.54060). Measurements were made at an angle of 2θ 5-50° with a step size of 0.017° and a step time of 10 s/step. The condition of divergence gap and the anti-scattering gap is 0.25° with a sample size of 10 mm.

#### *2.3.2. Differential scanning calorimetry (DSC)*

DSC analysis was performed using the Rigaku Differential Scanning Calorimeter 8230. The DSC was calibrated using high purity indium standards. A sample of about 2 mg was weighed and put into an airtight aluminum container, tightly closed with a press, and put into the DSC chamber. Measurements were carried out at a temperature of 30°C-200°C with a heating rate of 10°C/min under dry nitrogen gas at a flow rate of 50 mL/min.

#### *2.3.3. Fourier transform infrared spectroscopy (FTIR)*

The FTIR spectra were recorded using a Bruker Alpha Fourier Transform Infrared Spectrometer. The sample was placed on the FTIR sample board and tested with a resolution of 4 $cm^{-1}$ in the wavenumber range of 4000-400 $cm^{-1}$.

#### *2.3.4. Solubility test*

The solubility test was carried out using the shaking method. The sample was weighed in excess to produce a saturated solution (about 25 mg) and put into a 250 mL Erlenmeyer, and then added 25 mL of distilled water. Erlenmeyer was covered with aluminum foil and placed horizontally on the shaker. Shaking was carried out at 25°C at a speed of 150 rotations per minute for 24 hours then the supernatant was filtered using a 0.45 m cellulose nitrate filter membrane. The concentration of ketoprofen in the solution was determined using a UV-VIS spectrophotometer.

### 2.4. Evaluation of analgesic activity *in vivo*

An analgesic activity test was carried out using the writhing method. Experimental animals (male BALB/c mice) with healthy characteristics, 2-3 months old, the body weight of 20-30 grams, were divided into 5 groups with a total of 5 individuals in each group. Before treatment, the experimental animals were adapted to their environment for 7 days with a temperature of 25°C and a 12/12-hour light-dark cycle. Experimental animals were also given fasting treatment for 12-18 hours while still being given water. Experimental animals were grouped into control group (CMC Na 0.5%), group of the low dose of pure ketoprofen (3.25 mg/kg BW), group of high dose of pure ketoprofen (9.75 mg/kg BW), group of the low dose of ketoprofen-nicotinamide multicomponent solid (3.25 mg/kg BW) and a group of high dose of ketoprofen-nicotinamide multicomponent solid (9.75 mg/kg BW). The test sample was given orally to each experimental animal, and then the experimental animal was left for 50 minutes. Furthermore, each experimental animal was pain induced intraperitoneal with 0.5% acetic acid (0.2 ml/20g BW of mice). Observation of writhing in experimental animals was carried out for 90 minutes by counting the number of writhing every 5, 15, 30, 45, 60, 75, and 90 minutes. The response of writhing in experimental animals was characterized by stretching

the legs backward accompanied by contraction of the abdominal muscles that caused the stomach to stretch touch the table. The percentage of pain inhibition was calculated by the equation [(Wc-Wt)/Wc]x 100%, where Wc is the number of stretching of the control group and Wt is the number of stretching of the treatment group[7].

### 2.5. Acute toxicity test

An acute toxicity test was carried out on experimental animal groups by giving the test sample orally for 14 days. The groups of the experimental animals were the control group (CMC-Na 0.5%), group of the low dose of pure ketoprofen (3.25 mg/kg BW), group of high dose of pure ketoprofen (9.75 mg/kg BW), group of low dose of ketoprofen-nicotinamide multicomponent solid (3.25 mg/kg BW) and a group of high dose of ketoprofen-nicotinamide multicomponent solid (9.75 mg/kg BW). On the 15$^{th}$ day, each experimental animal was anesthetized, and then blood and stomach were taken. The blood of each experimental animal was determined for the levels of SGOT and SGPT, while the stomach was prepared for histological observation. Stomach were fixed in 10% buffered neutral formalin for 24 hours, and then sliced into small pieces. The stomach pieces are then immersed in alcohol for dehydration and in xylene for infiltration. The stomach sections were immersed in paraffin for embedding-blocking, and then sectioned using a rotary microtome with a thickness of about 5 microns. The preparations were then immersed in a solution of hematoxylin for 15 minutes and eosin for 4 minutes, and finally with xylene for 3 minutes. The preparations were dried and covered with a cover glass for observation under an optical microscope with a magnification of x200.

### 2.6. Statistic analysis

All data obtained are expressed in mean±SD. Statistical analysis was performed using the one way analysis of variance (ANOVA) test with a 95% confidence level. Statistical data processing was carried out using SPSS version 19 for Windows.

## 3. RESULTS AND DISCUSSION

### 3.1. Characteristics of multicomponent solids

The PXRD diffractograms of ketoprofen, nicotinamide, and ketoprofen-nicotinamide multicomponent solids are shown in Figure 1. The ketoprofen diffractogram showed specific 2θ diffraction peaks at 6.27, 14.33, 18.35, and 22.84°, while nicotinamide showed 2θ diffraction peaks specific at 14.68, 22.10, 25.77, and 27.20°. The diffractogram of the ketoprofen-nicotinamide multicomponent solid showed the pattern and intensity of the diffraction peaks differing from that of the constituent materials. These results indicate that the ketoprofen-nicotinamide solid has a different crystalline structure with each constituent material. The diffractogram of ketoprofen-nicotinamide multicomponent solid showed a similar pattern with the previous studies[4].

The DSC thermograms of ketoprofen, nicotinamide, and ketoprofen-nicotinamide multicomponent solids are shown in Figure 2. The DSC thermograms of ketoprofen and nicotinamide showed sharp endothermic peaks at 94.0°C (ΔH=92.443 J/g) and 129.2°C (ΔH=168.483 J/g) respectively, which indicates the melting point of the materials[8-9]. In the thermogram of the ketoprofen-nicotinamide multicomponent solid, it was seen that there was one endothermic peak at 64.7°C (ΔH=65.853 J/g). The presence of one endothermic peak indicated that ketoprofen and nicotinamide in the solid interact intermolecularly to form a new solid phase, namely the multicomponent solid of ketoprofen and nicotinamide.

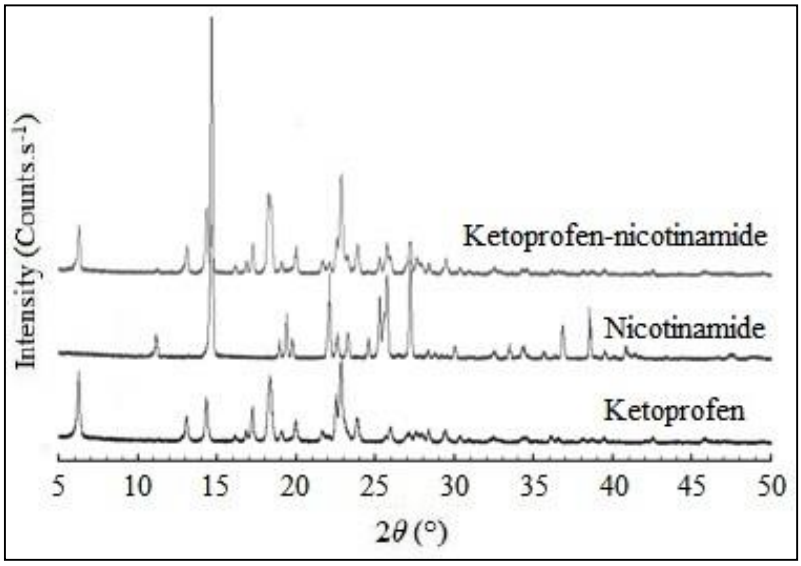


**Figure 1.** PXRD diffractograms of the constituent materials and multicomponent solids.

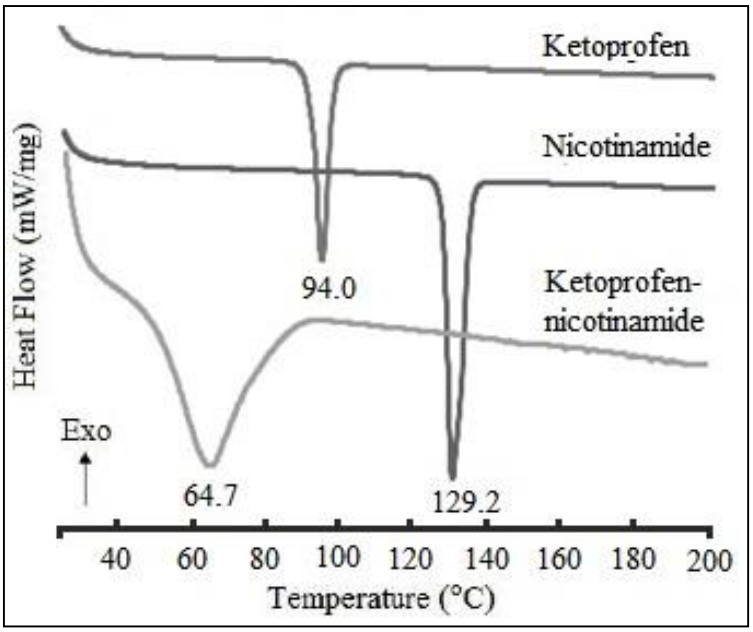


**Figure 2.** DSC thermograms of constituent materials and multicomponent solids. Multicomponent solids show a lower melting point than the constituent materials.

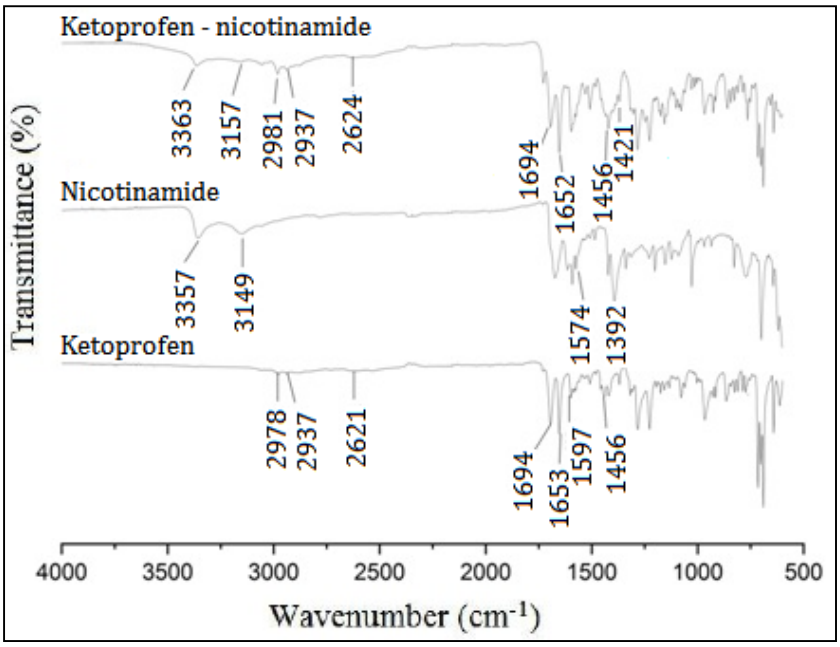


**Figure 3.** FTIR spectra of constituent materials and multicomponent solids.

The ketoprofen-nicotinamide multicomponent solid showed a lower melting endothermic peak than the starting material as mentioned in the literature[4].

The FTIR spectra of ketoprofen, nicotinamide, and ketoprofen-nicotinamide multicomponent solids are shown in Figure 3. The spectra of ketoprofen showed absorption peaks at 1694 $cm^{-1}$ (-C=O acid stretching), 1653 $cm^{-1}$ (-C=O ketone stretching), and 1597, 1575, and 1456 $cm^{-1}$ (-C=C- aromatic ring stretching). Nicotinamide has absorption peaks at 3357 $cm^{-1}$ (asymmetric-NH2 stretching), 3149 $cm^{-1}$ (symmetric-NH2 stretching), 1676 $cm^{-1}$ (-C=O amide stretching), 1614 $cm^{-1}$ (-NH deformation), and 1574 and 1392 $cm^{-1}$ (-CN stretching). The ketoprofen-nicotinamide multicomponent solid showed a shift in the absorption peaks compared to the absorption peaks of each component. This indicated the occurrence of intermolecular interactions between ketoprofen and nicotinamide molecules in the ketoprofen-nicotinamide multicomponent solids[4].

### 3.2. Solubility of multicomponent solids

The results of the solubility test of pure ketoprofen and ketoprofen-nicotinamide multicomponent solids in distilled water are shown in Figure 4. The solubility of pure ketoprofen and ketoprofen-nicotinamide multicomponent

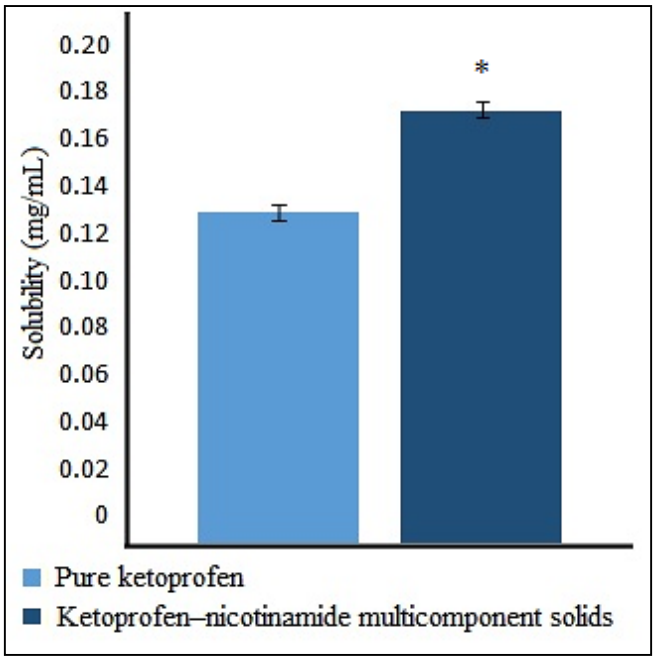


**Figure 4.** Solubility of pure ketoprofen and ketoprofen-nicotinamide multicomponent solids in distilled water (mean±SD, n=3). *: signifi-cant values compared to the pure ketoprofen, $p<0.05$.

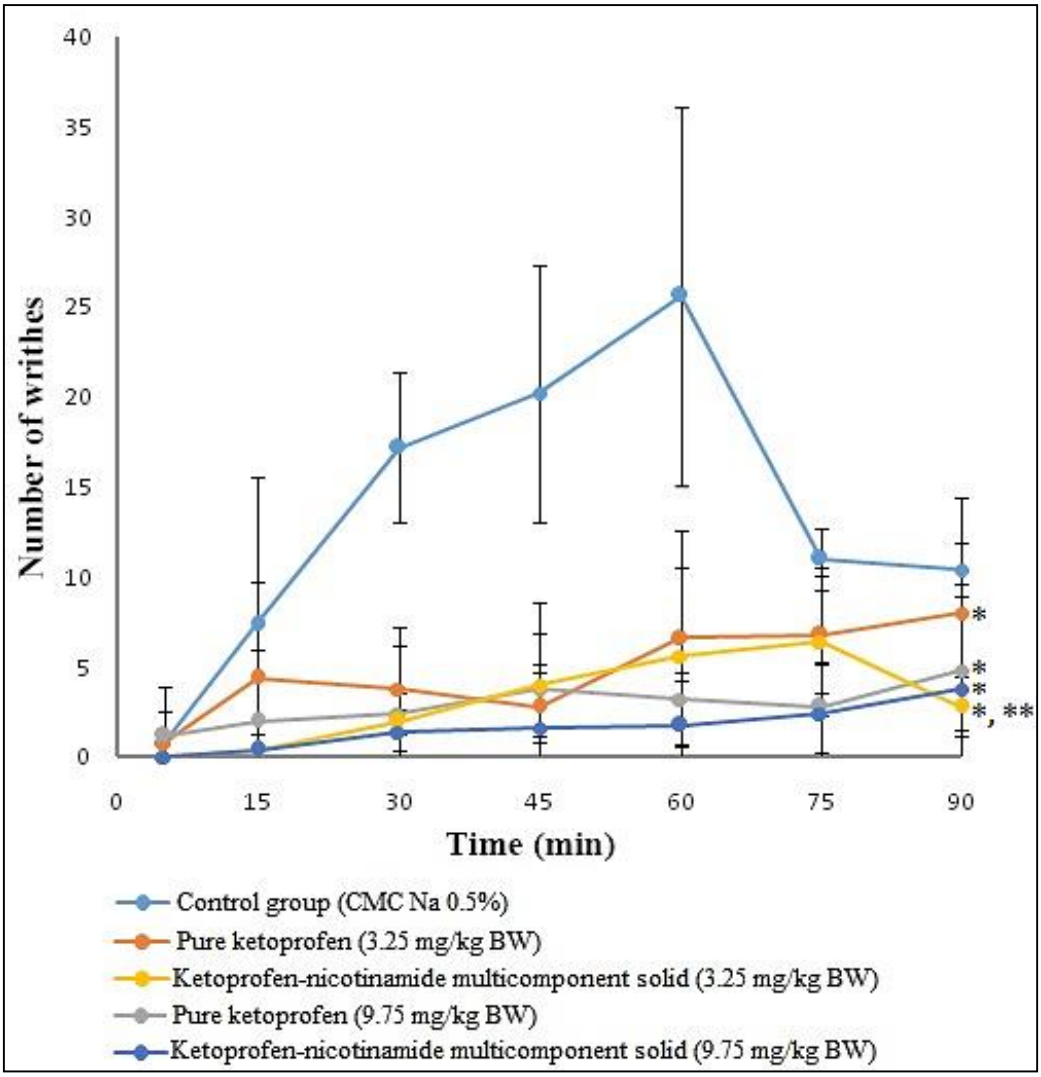


**Figure 5.** Number of writhing of experimental animals in analgesic activity test. Values are expressed in mean±SD (n=5). *: significant values compared to the control group, $p<0.05$ and **: significant values compared to the pure ketoprofen group, $p<0.05$.

solids is 0.141±0.00 and 0.184±0.01 mg/mL, respectively. These results indicated that the formation of the multi-component solids can increase the solubility of ketoprofen significantly ($p$<0.05). In the formation of multi-component solids, intermolecular interactions occurred between drug molecules and coformers, resulting in a new crystalline solid system with different crystalline packing from the initial components. The increase in solubility in multicomponent solids is caused by a decrease in the free energy of the crystal lattice in the new crystalline system so that the solid is more soluble in solvent[4,10].

### 3.3. Analgesic activity

The profile of the cumulative number of writhing of experimental animals for 90 minutes after administration of acetic acid 0.5% is shown in Figure 5. The number of the writhing of experimental animals in the control group showed an increase until the 60 minutes and then decreased. This indicated that CMC Na 0.5% has no analgesic activity so it didn't inhibit the pain response caused by administration of acetic acid 0.5%. The decrease in the number of writhing after the 60 minutes is thought to be due to the effect of pain due to administration of acetic acid 0.5% starting to weaken[11]. Overall, experimental animals in the control group (CMC Na 0.5%) showed the highest number of writhing compared to the other treatment groups.

Based on the number of writhes of the experimental animals at the 60 and 90 minutes, the percentage pain inhibition could be calculated so that the data obtained as shown in Table 1. At the 60 minutes, the low-dose and high-dose ketoprofen-nicotinamide treatment groups didn't show an increase in the percentage of pain inhibition significantly ($p$>0.05) compared to the pure ketoprofen treatment group. However, at 90 minutes, the low-dose ketoprofen-nicotinamide treatment group (3.25 mg/kg BW) showed a significant increase in the pain inhibition percentage ($p$<0.05) compared to the pure ketoprofen treatment group. These results indicated that the formation of ketoprofen-nicotinamide multi-component solids can enhance the analgesic effect of ketoprofen in experimental animals. The increase in the analgesic effect of ketoprofen-nicotinamide multicomponent solids is thought to be due to an increase in the solubility of ketoprofen so that the absorption and bioavailability of ketoprofen from multicomponent solids become better[12].

### 3.4. Acute Toxicity

SGOT and SGPT levels in the blood are indications of pathological changes in vital body organs such as the liver, kidneys, pancreas, and liver so that they can be used to evaluate the safety and toxicity of a drug[13]. The levels of SGOT and SGPT of the experimental group are shown in Table 2. Experimental animals in the control group (CMC Na 0.5%) showed SGOT and SGPT levels of 150.57±34.89 and 36.74±6.26 IU/L, respectively. Experimental animals with the treatment of ketoprofen (pure form or multicomponent solid) showed a range of SGOT and SGPT levels of 196.46±41.87-264.30±130.77 IU/L and 37.42±9.07-70.95±4.96 IU/L, respectively. The SGOT and SGPT levels in the ketoprofen treatment group did not show a significant difference with the control group ($p$>0.05) and were still classified as normal levels for SGOT (54-298 IU/L) and SGPT (15-84 IU/L)[14-15].

Observations on the stomach histology of the experimental animals are aimed at describing the damage to the gastric organs due to the administration of test samples. The histology of the stomach of experimental animals is shown in Figure 6. Stomach histology of the control group (CMC Na 0.5%) showed layers of the

**Table 1.** The number of writhes and percentage of pain inhibition of the experimental animals at the 60 and 90 minutes (mean±SD, n=5).

| Groups | Number of writhes | | Percentage of pain inhibition (%) | |
|---|---|---|---|---|
| | 60 minutes | 90 minutes | 60 minutes | 90 minutes |
| Control (CMC Na 0.5%) | 71.2 ± 15.80 | 92.6 ± 16.10 | 0.00 ± 0.00 | 0.00 ± 0.00 |
| Pure ketoprofen (3.25 mg/kg BW) | 18.4 ± 7.43 | 33.2 ± 5.85 | 71.9 ± 15.60* | 63.9 ± 3.32* |
| Pure ketoprofen (9.75 mg/kg BW) | 12.6 ± 12.10 | 20.2 ± 10.90 | 83.1 ± 13.90* | 79.1 ± 8.81* |
| Ketoprofen-nicotinamide (3.25 mg/kg BW) | 12.0 ± 7.31 | 21.2 ± 9.44 | 83.5 ± 7.01* | 77.4 ± 7.74*, ** |
| Ketoprofen-nicotinamide (9.75 mg/kg BW) | 5.2 ± 6.14 | 11.4 ± 8.82 | 93.2 ± 7.72* | 88.5 ± 7.60* |

*Significant values compared to the control group, $p$<0.05
**Significant values compared to the pure ketoprofen group, $p$<0.05

**Table 2.** The levels of SGOT and SGPT of the experimental animals after 14 days treatment (mean±SD, n=5).

| Groups | Levels of SGOT±SD (IU/L) | Levels of SGPT±SD (IU/L) |
|---|---|---|
| Control (CMC Na 0.5%) | 150.57 ± 34.89 | 36.74 ± 6.26 |
| Pure ketoprofen (3.25 mg/kg BW) | 229.31 ± 94.98 | 57.34 ± 15.53 |
| Pure ketoprofen (9.75 mg/kg BW) | 264.30 ± 130.77 | 70.95 ± 4.96 |
| Ketoprofen-nicotinamide (3.25 mg/kg BW) | 196.46 ± 41.87 | 37.42 ± 9.07 |
| Ketoprofen-nicotinamide (9.75 mg/kg BW) | 221.28 ± 62.24 | 47.91 ± 12.84 |

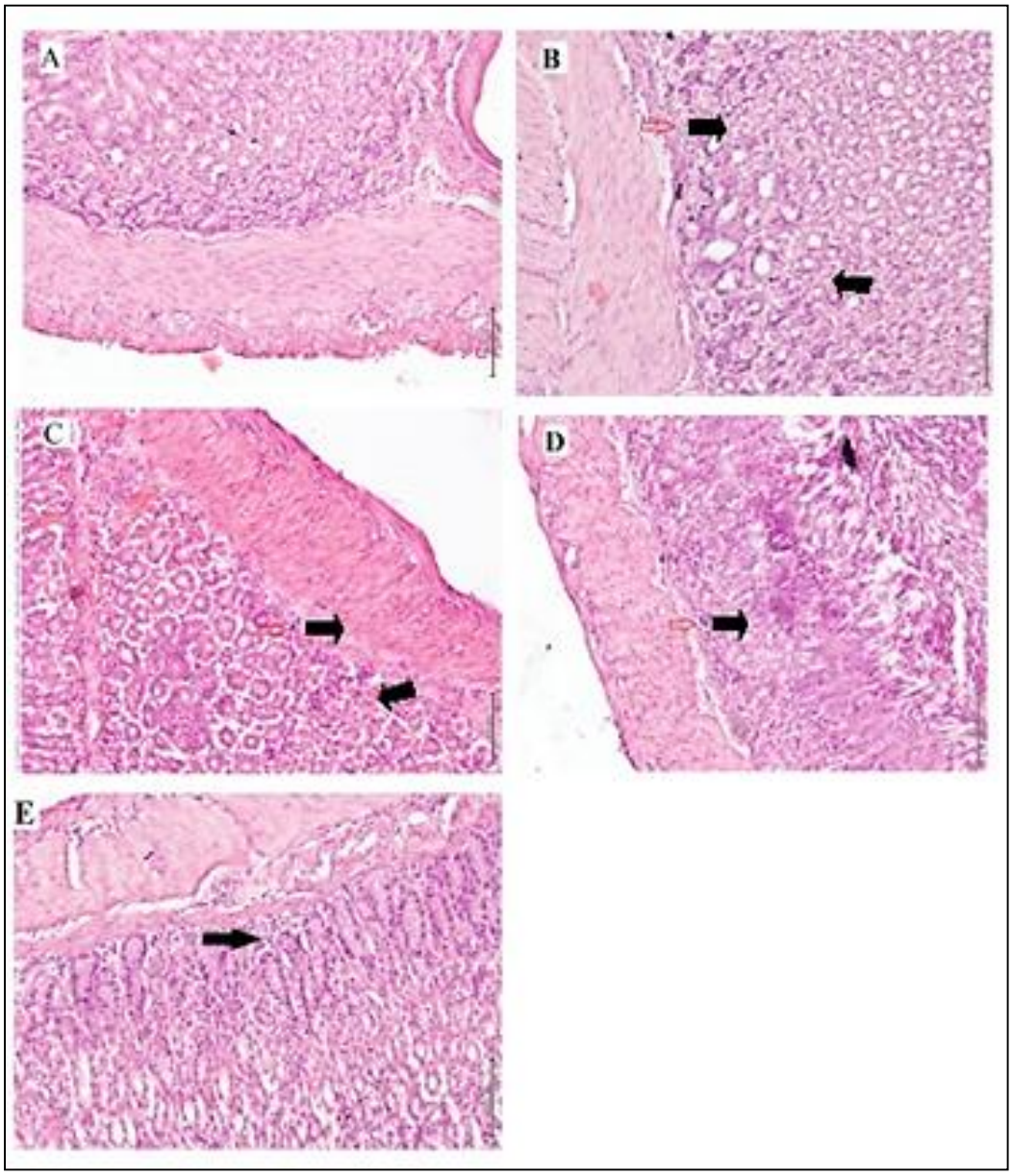


**Figure 6.** Stomach histology of experimental animals after 14 days treatment (a) control group (CMC-Na 0.5%), (b) pure ketoprofen 3.25 mg/kg BW, (c) pure ketoprofen 9.75 mg/kg BW, (d) ketoprofen-nicotinamide multicomponent solid 3.25 mg/kg BW, and (e) ketoprofen-nicotinamide multicomponent solid 9.75 mg/kg BW. Marking indicates the presence of infiltration of neutrophil inflammatory cell. (Hematoxylin & Eosin staining. x200).

stomach consisting of mucosa, submucosa, muscularis externa, and serosa. The stomach linings of the experimental control group looked normal and intact, no infiltration of inflammatory cell was found. In the pure ketoprofen group at doses of 3.25 and 9.75 mg/kg BW, there was a change in the stomach lining in the form of infiltration of neutrophil inflammatory cells. The infiltration of inflammatory cell was spread over the mucosa and submucosa but no erosions and ulcerations were seen. These results indicated that the administration of ketoprofen to experimental animals for 14 days causes stomach histology to experience a mild gradation as an indication of acute gastritis[16-17]. In the stomach histology of experimental animals, the ketoprofen-nicotinamide multicomponent solid group at doses of 3.25 and 9.75 mg/kg BW also showed changes in the stomach lining, but the infiltration of neutrophil inflammatory cells was lighter when compared to the pure ketoprofen group. This is thought to be because nicotinamide in the multicomponent solids can play a role in cell DNA repair through an inhibitory mechanism of poly (ADP-ribose) polymerase (PARP) thereby reducing inflammatory cell infiltration caused by ketoprofen[18].

## 4. CONCLUSION

The formation of ketoprofen-nicotinamide multicomponent solids can increase the solubility of ketoprofen significantly ($p<0.05$) compared to pure ketoprofen. In the experimental animals, ketoprofen-nicotinamide

multicomponent solid at a dose of 3.25 mg/kg BW showed a significant ($p$<0.05) increase in pain inhibition compared to the pure ketoprofen. Treatment of ketoprofen to experimental animals (pure and multicomponent solids) did not show a significant ($p$>0.05) increase in the levels of SGOT and SGPT when compared to the control group. Observations on stomach histology of experimental animals showed that administration of ketoprofen-nicotinamide multicomponent solids at doses of 3.25 and 9.75 mg/kg BW gave lighter neutrophil inflammatory cell infiltration when compared to the pure ketoprofen.

## 5. ACKNOWLEDGEMENT

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### Conflict of interest

The results of this article are not in conflict with the interests of the authors.

### Funding

None to declare.

### Ethical approval

All procedures for using experimental animals have been approved by the Research Ethics Committee of the Faculty of Dentistry, University of Jember (Animal protocol number: 1038/UN25.8/KEPK/DL/2020).

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