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Cycloxygenase(COX-1,COX-2) Inhibitor Induced Gastrointestinal abscess– As Ramification of Adverse Impact of NSAIDs

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ARTICLE DETAILS

Research Paper

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ABSTRACT

The pain and inflammation brought on by arthritis and other musculoskeletal conditions use non-steroidal anti-inflammatory medications (NSAIDs) for treatments. Serious adverse effects, some potentially fatal, are a common side consequence of NSAIDs. NSAIDs may have unintended side effects when taken alongside other medications. Additionally, it is commonly recognized that indomethacin severely harms the mucosa of the stomach. Hemorrhage, ulcerative lesions, and severe mucosal erosions can all result from indomethacin-induced mucosal injury. COX inhibition reduces mucosal secretion, reduces microcirculation, and hinders mucosal blood flow to the stomach. Reduced secretion makes it more difficult for the body to defend against acid that is triggered by diet.

INTRODUCTION: NSAID-induced injury to the stomach mucosa primarily results from a local and/or systemic action. Intercellular adhesion molecule-1 is released by NSAIDs taken orally from the vascular endothelial cells that line the stomach mucosa. Due to this mechanism, inflammatory cytokines including interleukin 1 (IL-1) and tumour necrosis factor α (TNF-α) cause large numbers of neutrophils to adhere to vascular endothelial cells. Additionally, the reactive oxygen species that neutrophils are generates, Prostaglandins, particularly Prostaglandin E2 and Prostaglandin L2, are continuously produced by the gastric mucosal portion that are essential for maintaining mucosal integrity and shielding it from harmful substances. Prostaglandins have been shown to interact with nearly every mucosal defense mechanism. Notably, they may increase the generation of bicarbonate and mucus while

decreasing acid output.Mucus and the bicarbonate barrier, which are components of the Superficial Gel layer in the G.I. tract, are the initial line of defense for the stomach mucosa. Mucus gel and bicarbonate anions combine to produce a layer that covers the surface of the gastric mucosa. The layer can hold on to the bicarbonate ions released by the epithelial cells on the surface and keep the pH close to 7 when it comes to the mucosa. The next line of defense for the mucosa is the continuous layer of surface epithelial cells joined by tight junctions. Tight connections allow epithelial cells to establish an impermeable barrier that stops pepsin and gastric acid from invading the stomach and damaging the deeper layers of the gastric lining. Mucosal progenitor cells have capabilities for cell renewal process, that preserves gastric epithelial cells on the surface continuous layer,While the process of epithelial renewal typically takes three to seven days, the migration of preserved cells from the neck region of the gastric gland is what allows the epithelium to regenerate in a matter of minutes after being exposed to harmful substances. Additionally, prostaglandins (PGE2) and gastrin interact with EGFR. After prolonged use of nonsteroidal anti-inflammatory drugs (NSAIDs), such as aspirin, which is combined with prescription and over-the-counter painkillers such as ketoprofen, naproxen like medicines, ibuprofen (Advil, Motrin IB, and others), and others, the lining of the stomach and small intestine may undergo erosion or become inflamed .Osteoarthritis patients and older adults who frequently take these medications for pain have a higher risk of developing stomach ulcers.

Methodology:

Experimental animals

For the aim of the experiment, old male adult albino rats (wistar strain) weighing between 130 and 150 grams at eight weeks of age were used. The rats were gathered seven days before the experiment in Serampore College's animal house. The animals were kept in cages with typical laboratory settings, which included a 12-hour light-dark cycle, a temperature of 25±2¬ºC, and a humidity of 55±5%. A regular diet of 71% carbohydrates, 18% protein, 7% fat, and 4% salt mixture was fed to the animals. Throughout the whole trial, food and water were provided for each and every rat. Daily cleaning and the removal of excrement and spilled food from the cages helped to maintain good hygiene.

Experimental Design:

The five groups of five experimental rats each were randomly assigned, and the animals were handled as follows:

Throughout the experiment,

Group 1 (Control): 5 animals of a cage were subjected to control group, delivered normal oral administered saline (10 ml/kg body weight/day).

Group 2(Indomethacin treated group): On the day of sacrifice, rats were treated with 30 mg/kg of oral Indomethacin (NSAIDs), a cox-1 and cox-2 inhibitor analgesic medication.On the day of sacrifice, a 15 mg/kg dosage of indomethacin was used to create a stomach abscess or erosion. Six hours after ,the animals were treated with indomethacin, were sacrificed. Following this, the rats' stomachs were removed, sliced open along with their greater curvature, and cleaned in regular saline. After that, it was leveled, and the quantity and severity of erosions were tallied.

Macroscopic assessment of the stomach

The stomachs were opened by means of larger curvature, cleaned with water to get rid of any remaining food particles and blood clots, and then looked via a 10× magnifying lens to see if any ulcers had formed. They counted the amount of ulcers. The ulcer was scored in the following ways:

- Normal stomach color (0),
- Red coloration (0.5),
- Hemorrhagic injury (1.5),
- Spot abscess(1),
- Deep lesion (2)
- Gastric Mucosal Perforation(3)

Measurement was carried out for the total area of gastric abscess and mucosa.

- **Estimation of pH**

The G.I pH of the solution was measured by pH Meter, an aliquot of gastric juice of 1 ml was diluted with 1 ml of distilled water [Dashputre, 2011].

- **Calculation of Total Acidity**

An aliquot of 1 ml of gastric juice that had been diluted with 1 ml of distilled water was placed into a 50 ml conical flask. That was followed by the addition of two drops of phenolphthalein indicator, and this combination was titrated with 0.01N NaOH until a persistent pink color was seen. The

overall acidity was calculated using the following formula [Dashputre 2011], which was expressed as mEq/L [pH = VNaOH×N×100mEq/L0.1[where N is normality and V is volume]].

Preparation of Gastric Tissue homogenate:

The glandular regions of the stomach tissue are removed, and the stomach is then washed with ice-cold normal saline. Using a homogenizer and ice-cold phosphate-buffered saline (0.1mol/L) with a cocktail of mammalian protease inhibitors, half of the stomach was homogenized. The homogenates were centrifuged for 15 minutes at 4°C and 2500 rpm, following which the supernatant was used to measure the biological activities of the stomach homogenate. The stomach homogenate was subjected to bioassaying.

Calculating the Production of Nitric Oxide (NO)

Since nitric oxide breaks down quickly in aerated solutions to produce stable nitrite/nitrate products, nitrite buildup was measured in the current work as an indicator of NO generation using the Griess reaction (Raso et al., 1999). The amount of nitrite (micromolar unit) in the mixture as trial and calculated via Standard curve of Sodium Nitrate.

Lipid peroxidation Estimation

Lipid peroxidase function was assessed by measuring the amount of malondialdehyde (MDA), a marker of lipid peroxidation. Following the thiobarbituric acid (TBA) test, lipid peroxidation was quantitatively quantified (Wills, 1987). The amount of MDA produced was quantified using TBA. The information was shown as nanomoles of MDA for each milligram of protein.

Calculating of Superoxide Dismutase (SOD)

The SOD activity was measured using the Nitroblue tetrazolium (NBT) method, which is based on SOD's suppression of NBT reduction (Sun et al., 1988). Next, SOD activity was converted from the relative absorbance value to a protein unit of (/mg) SOD activity.

Determination of Catalase (CAT) activity

By tracking the disintegration of H2O2 at 240 nm, catalase activity was determined using the Aebi (1984) method. The absorbance difference per unit of time was used to calculate the CAT activity. U/mg was the unit of protein used to express the values.

Calculating the concentration of Reduced Glutathione (GSH)

The GSH level was measured using 5,5'-dithiobis-2-nitrobenzoic acid (DTNB). The absorbance of reduced chromogen was observed at 412 nm using spectrophotometry. With the aid of a standard curve, the GSH level was calculated and reported as mM/mg protein (Ellman, 1959).

Calculation of the Protein

The amount of protein in the stomach tissue was measured using the Lowry et al. (1951) method, which employed bovine serum albumin (BSA) as a standard.

Determination of Cytokines Levels.

The commercially available ELISA kit for rats, provided by Raybiotech (USA), was used to quantify the serum levels of IL-6 and TNF-α. Every sample was analyzed twice. The intra-assay variance for TNF-α and IL-6 were 5.7% and 6.3%, respectively..

Examination of Intestinal motility:

Water was available to albino wister rats (130–150g) throughout a 6-hour fast. The animals in the experimental group were given a 10% active charcoal suspension in water. The animals were slaughtered and their guts removed after thirty minutes. Measurements were made of the small intestine's overall length and the charcoal suspension's travel distance (D.W et al., 1959).

- **HISTOPATHOLOGICAL ANALYSIS:**

The stomach tissue was removed from Experimental animals of both control group and the Indomethacin injected rats and tissues are fixed in 10% formaline solution for at least 48 hrs. These were next processed routinely and the section of tissues was implanted in paraffin wax. Histological sections were cut with 5-6µm and routinely stained with Haemotoxylin and Eosin.

- **Statistical analysis**

The data was presented as Mean±SE. The Kruskal-Wallis nonparametric ANOVA test was used to determine whether there was a significant difference in the scores of the various groups. The Mann-Whitney U multiple comparison test was used to determine the correlation between the research variable and test for inter-group significant differences. The statistical analysis was conducted using StatsDirect 2.7.2. If P was less than 0.05, differences were deemed significant.

- **Results :**

Parameters	Control	IND
pH of gastric content	5.92±0.40	3.21±0.44[a]***
Gastric acid volume (ml/4h)	1.14±0.19	2.90±0.23[a]***
Titrable acid (mEq/L)	13.2±3.96	27.6±2.60[a]***
Acid output (µEq/h)	3.76±0.76	20.01±1.98[a]***
Gastric abscess indexIndex	0.00±0.00	10.0±1.58[a]***
Gastrointestinal transit ratio (%)	66.44±5.32	42.88±3.45[a]***

Significance level based on Mann-Whitney test - *: P<0.05, **:P<0.01, ***: P<0.001. NS: Not significant

Ulcer lesion Area	INDOMETHACIN
Ulcer lesion area(mm^2)	40.34±3.69

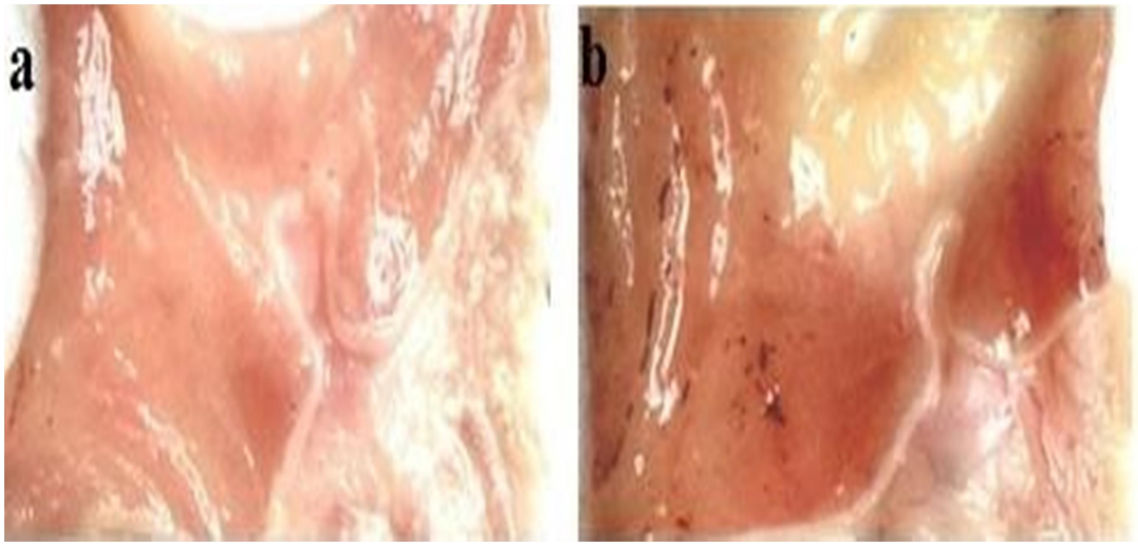

Fig 1 :Macroscopic assessment of the stomach mucosa in (a) Control: revealed no mucosal damage. (b) After receiving Indomethacin, the stomach mucosa had significant hemorrhagic bands, a lesion, and a hole.

Estimation of Nitric oxide(NO) production

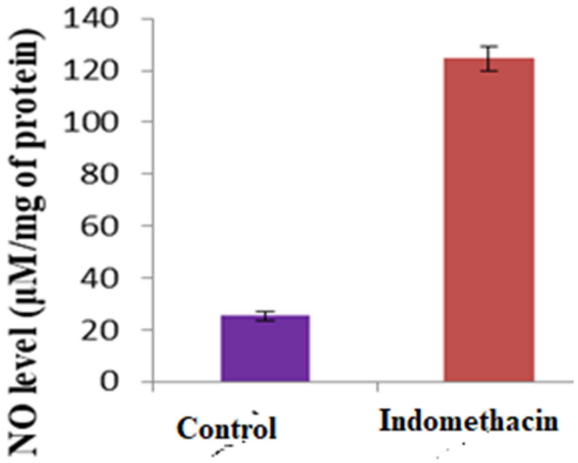

Fig 2: The impact of oral indomethacin (20 mg/kg) on male rats' nitric oxide (NO) levels. The mean (±S.E., n = 5) is displayed in the error bar. The significance, according to the Kruskal-Wallis test, is P<0.001. significance of the Mann-Whitney U multiple comparison test: Indomethacin against control (P<0.001);

ESTIMATION OF LIPID PEROXIDATION LEVEL

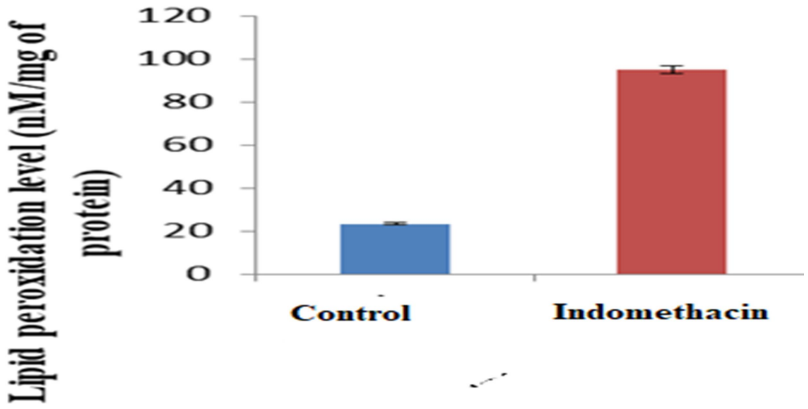

Fig 3: The Impact Of Oral Indomethacin (20 mg/kg) on male rats' Lipid Peroxidation levels. The mean (±S.E., n = 5) is displayed in the error bar. The significance, according to the Kruskal-Wallis test, is P<0.001. significance of the Mann-Whitney U multiple comparison test: indomethacin against control (P<0.001);

ESTIMATION OF SUPEROXIDE DISMUTASE (SOD) ACTIVITY:

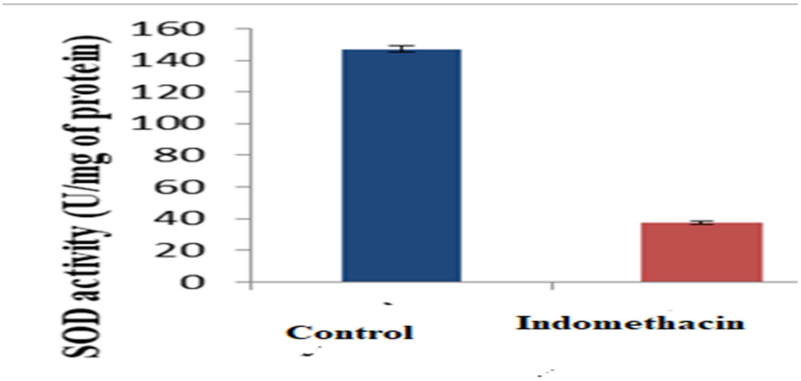

Fig 4: In male rats, the oral administration of 30 mg/kg of indomethacin changed the activity of the enzyme superoxide dismutase (SOD). Shown is the mean±S.E.(n=5) on the error bar. The significance, according to the Kruskal-Wallis test, is P<0.001. Indomethacin vs. Control: Significance of the Mann-Whitney multiple U comparison test (P<0.001)

Estimation of Catalase Activity:

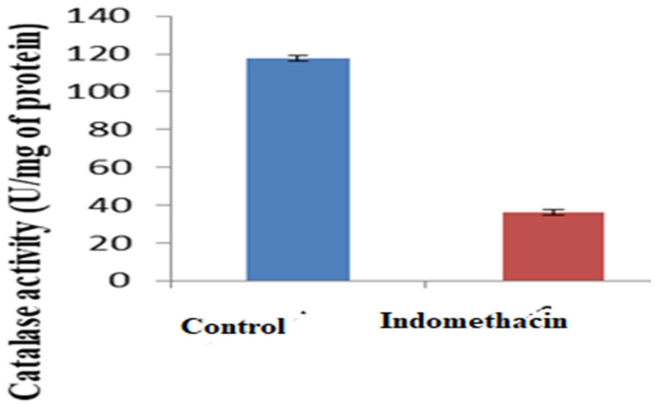

Fig 5:Indomethacin (30 mg/kg orally) altered the male rat's catalase activity. The error bar shows the mean±S.E.(n=5). Based on the Kruskal-Wallis test, the significance is P<0.01. Mannn-Whitney U multiple comparison test significance: indomethacin vs. control (P<0.001);

Estimation of Reduced Glutathione (GSH) level:

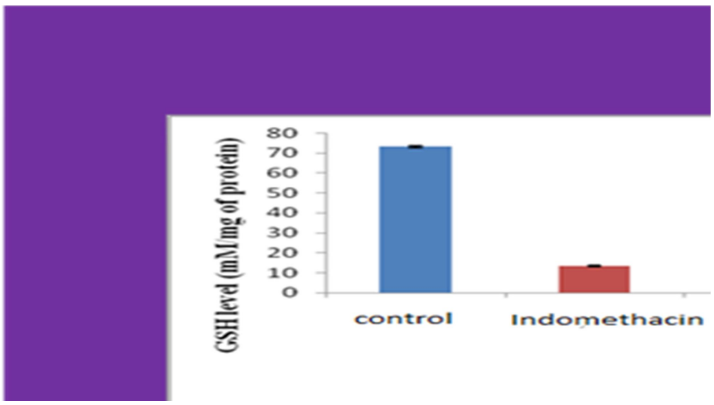

Fig 6: The Impact of 30 mg/kg oral indomethacin on the lowering of glutathione level (GSH) activity in male rats. Shown is the mean±S.E.(n=5) on the error bar. P<0.05 for the significance level of the Kruskal-Wallis test. Mann-Whitney significance of the U multiple comparison test: Indomethacin against control (P<0.001);

Determination of TNF-α:

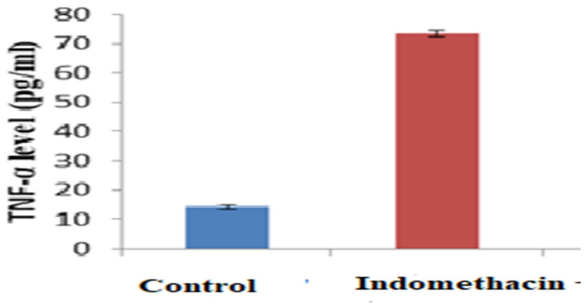

Fig :7 When male rats were administered 30 mg/kg of indomethacin orally, their levels of TNF-α changed. Shown is the mean±S.E.(n=5) on the error bar. P<0.05 for the significance level of the Kruskal-Wallis test. Mann-Whitney significance of the U multiple comparison test: indomethacin against control (P<0.001);

Determination of IL-6

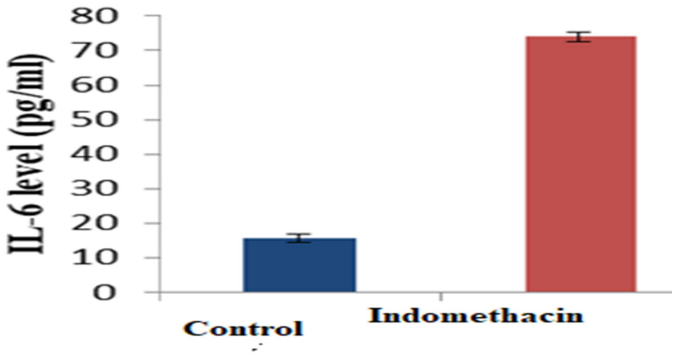

Fig:8; The cytokine level (IL-6) in the male rat was changed by an oral dose of indomethacin (30 mg/kg). Shown is the mean±S.E.(n=5) on the error bar. P<0.05 for the significance level of the Kruskal-Wallis test. Mann-Whitney Domethacin vs. control: significance of the U multiple comparison test (P<0.001)

Histopathological Assay

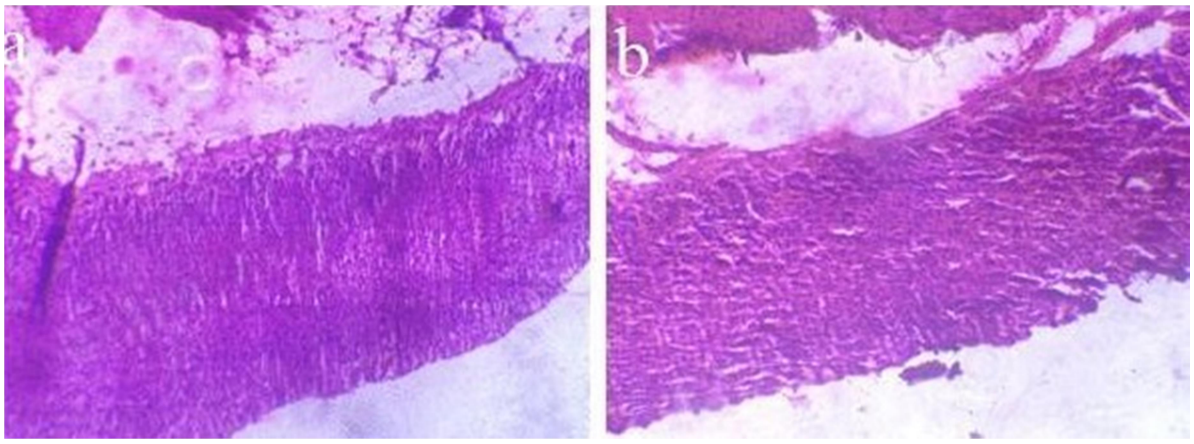

Fig 9: Histological evaluation of gastric mucosa of (a) Control: showed no injury to the gastric mucosa. (b) Indomethacin treated: showed remarkably severe disruption to the gastric mucosa

➢ Discussions

The indomethacin-generated ulcer model. When indomethacin is taken orally, it disrupts the mucosa barrier in the stomach, altering the vasculature and producing regional damage (fig:1). As a crucial first line of defense, mucus discharge keeps stomach tissue from deforming and avoids direct contact with the digestive enzymes. Indomethacin caused bleeding lesions, mucosal friability, and extensive submucosal edema by damaging the epithelial cells of the stomach layer. Indomethacin decreases the concentration of proteins by destroying epithelial cells.NSAIDs inhibit the enzyme cyclooxygenase and decrease the synthesis of prostaglandin (PG), a molecule that coats and shields the mucosa of the stomach. Furthermore, gastrointestinal damage results from NSAIDs' direct stimulation of inducible NO synthases (iNOS, NOS2) to create excessive NO.It increases levels of proinflammatory cytokines.When indomethacin is taken orally, it disrupts the mucosa barrier in the stomach, altering the vasculature and producing regional damage (fig:1). As a crucial first line of defense, mucus discharge keeps stomach tissue from deforming and avoids direct contact with the digestive enzymes. Indomethacin caused hemorrhagic wounds, mucosal friability, severe submucosal edema, and caustor. SOD levels and catalase activity are lowered by cytokines such as TNF-alpha and IL-6.It was followed by a significant halt to the decrease in the protein content. Stomach acid buildup promotes autodigestion, the breakdown of the gastric mucosa barrier, decreased gastric blood flow, and finally the development of ulcers.. When the stomach's tissue folds flatten, a wider area is created that can interact with substances. Additionally, a decrease in stomach motility results in a decrease in the percentage of gastric mucosal damage. as a

result of its potential connection to the reduction of stomach motility.Oxidative stress causes tissue to create superoxide and hydroxyl radicals, which play a crucial role in the onset of disease. As a result, eliminating these radicals is a helpful precaution against injury. Antioxidant enzymes such as SOD and CAT, as well as regulating thWhen the stomach's tissue folds flatten, a larger area is created that can interact with substances. Moreover, as reduced stomach motility is associated with increased lipid peroxidation and the inhibition of gastric motility, the percentage of gastric mucosal damage falls as stomach motility declines. In rats given indomethacin, the gastric tissue homogenate showed increased NO generation (fig. 2), increased MDA levels (fig. 3), and decreased SOD (fig. 4) and CAT (fig. 5) activity. Can both rapidly transform the peroxyl radical into physiologically safe molecules, such as water, and so help prevent ulcers. because of changes in the stomach tissue's shape and biochemistry brought on by cell membrane injury . These results imply that tissue integrity can be preserved by employing lipid peroxidation to stop the degradation of membrane phospholipids. GSH is one of the materials required to maintain cellular integrity (Sokkary et al., 2007). In the current study, the levels of GSH deposited in the stomach tissue were significantly decreased by the injection of indomethacin.The combination of TNF- and IL-6 stimulates endothelial activation, which keeps neutrophil recruitment going during inflammation. The antiulcerogenic action of indomethacin is therefore linked to a reduction in TNF-α and IL-6 in the model.

References

Alcaide.,Azcutia, V, Bu , D.X E N., Grabie, P., Griffin, G.K., Luscinskas ,Maganto-Garcia, (2012) IL-17 and TNF-a sustain neutrophil recruitment during inflammation through synergistic effects on endothelial activation. The Journal of Immunology 188, 6287–6299

Alp H ,Polat B, Suleyman H,.(2010) Adaptation of rat gastric tissue against indomethacin toxicity. ChemBiol Interact. ;186:82–89.

Amagase, Okabe, S.,K., (2005). An overview of acetic acid ulcer models—the history and state of the art of peptic ulcer research. Biological and Pharmaceutical Bulletin 28: 1321–1341.

A.M ,Beserra,., Balogun, Calegari, Dos Santos ,J.C,Lima, M.C,P.I., R.M., Silva, S.O., Martins, Souza.,(2011). Gastroprotective and ulcer-healing mechanisms of ellagic acid in experimental rats. Journal of Agricultural and Food Chemistry. 59: 6957–6965.

Anand TJ, Ali SKL, ManjulaRR,Padmaja V Sai MH(2013) . Anti-ulcer activity of *Physalis minima* plant extract in albino rats. *Int J Pharm SciRes*; 4(12): 4615-18. doi: 10.13040/IJPSR. 0975-8232.4(12).4615-18

Andrade, , B.K ,Clasen, F de, Gandolfi., Gimenez, J.C, Lemos, M.A. Filho, S.F,R.B ,Ticona, , V.C., Zanatta, (2009). Gastroprotective activity of alkaloid extract and 2-phenylquinoline obtained from the bark of Galipealongiflora Krause (Rutaceae). Chemico-Biological Interactions 180, 312–317.

Dashputre NL, Naikwade NS.(2011) Evaluation of anti-ulcer activity of methanolic extract of *Abutilon indicum* Linn leaves in experimental rats. Int J Pharm Sci Drug Res. ;3(2):97–100