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INVESTIGATE THE EFFECT OF BIOCHANIN-A ON GASTRIC ULCER AND COLITIS INDUCED BY INDOMETHACIN IN EXPERIMENTAL RATS

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ABSTRACT:

Background: Biochanin-A is a notable bioactive compound abundantly present in many traditional medicinal plants. It is used as antioxidant, anti-inflammatory, antispasmodic and anticancer agents.

Objectives: The purpose of this study was to investigate the effects of Biochanin-A on gastric ulcer and colitis induced by Indomethacin in experimental rats.

Methods: Rats (150-200g) were randomly divided into four groups each containing five rats (n=5). Group-I (normal control) rats were given 10 mL/kg, body weight **(**1% CMC) orally for five days. Group-II (Disease control) rats were given Indomethacin as an inducing agent at a dose of 10 mg/kg, body weight orally for 2 consecutive days. Group-III (Self-healing group) rats were given Indomethacin orally as an inducing agent at a dose of 10 mg/kg body weight for 2 consecutive days and 1% CMC was administered for the next 5 days. Group-IV (treatment group) rats were given Indomethacin at the dose of 10 mg/kg, orally for 2 consecutive days followed by administration of Biochanin-A at a dose of 10 mg/kg, orally for 5 days. After the completion of treatment duration, rats were sacrificed using a high dose of anesthesia, stomach was cut and opened along with the greater curvature and washed with distilled water with care. In the same manner colons were also cut and removed and washed.

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Both stomach and colon were examined for lesions. After the examination, the stomach and colon were weighed and the stomach was immediately immersed in alcian blue solution to determine the mucus wall thickness in gastric tissue and other ulcer parameters were assessed. Gross anatomical and histo-pathological changes in stomach and colon were also evaluated.

Result: The present study showed that normal control group the (i.e. Group I) which was fed only with the basal diet along with CMC produced normal levels of mean ulcer index, mean gastric wall mucus thickness and weight/length ratio. It was observed that Indomethacin (10 mg/kg, p.o)) treated rats (i.e group II) showed significant ($P<0.01$) increased levels of mean ulcer index and significant decreased in mean gastric wall mucus thickness as well as weight/length ratio compared to the normal control rats (i.e. group I). Treatment with Biochanin-A (10mg/kg,p.o.) causes significant reduction in mean ulcer index and increase in gastric mucus content as well as weight/length ratio compared to self-healing group and indomethacin treated group.

Conclusions: Our result suggested that treatment with Biochanin-A exhibited gastro-protective effect in Indomethacin induced gastric ulcer in experimental rats as evidenced by the strengthening of the gastric mucosa, decrease in ulcer index and increase of the gastric wall mucus.

Keywords: Biochanin- A, Indomethacin, Ulcer index, Antiulcer, Ulcerative Colitis, Ranitidine

INTRODUCTION

1.1 Peptic ulcer disease

Peptic ulcer disease is a global crisis through a significant occurrence in India. Numerous depth studies from various sections of the country estimate that it occurs in four to ten people per thousand. Jammu & Kashmir, Tamil Nadu, and Andhra Pradesh are three states in India that are regarded as being extremely dangerous. Although there is no known cause of peptic ulcers, the disease causes chronic pain, lost work hours, and even death. Smoking, drinking, and spices all contribute to the progression of the condition and frequently induce major ulcer complications (Tendon et al., 2003).

In the United States, 350,000 new cases of peptic ulcers (duodenal and stomach) are diagnosed each year, affecting around 4 million people. The chance of having a peptic ulcer in one's lifetime is roughly 10% for males and 4% for women in the United States. Children and adolescents can also develop peptic ulcers, which are reversible, relapsing lesions that

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often affect middle-aged to older adults. After a period of weeks to months of active disease, they frequently happen without evident triggering factors and may be later healing with or without therapy. The possibility of recurring infections with H. pylori contributes in part to the risk of developing peptic ulcers even after they have healed. The male to female ratio for duodenal ulcers is approximately 3:1, but the ratio for stomach ulcers is between 1:5 and 2:1. Most women experience symptoms during or after menopause (Cotran et al., 2000).

In the past two decades, a number of innovative drugs for the treatment of gastric and duodenal ulcers have been developed. Recent research has also contributed significantly to our understanding of mucosal protection mechanisms and their role in the healing of the upper gastrointestinal tract from acute damage during this time period (Parmer et al., 1993).

Over the past few decades, there has been abundance in research with the goal of developing effective and safer anti-ulcer medications, both synthetically and from natural resources. According to research on the clinical assessment of synthetic medications, recurrence and drug interactions are possible during ulcer therapy. In order to find innovative molecules that offer improved protection and lower the chance of recurrence, the search for the ideal anti-ulcer drug continues, with a focus on botanicals (Goal et al,. 2002).

1.2 Ulcerative colitis

A number of signaling molecules, such as pro-inflammatory and anti-inflammatory mediators, mediate the complex pathos-physiological process of inflammation in bodily tissues. Cohn's disease (CD) and ulcerative colitis (UC) are two common inflammatory bowel diseases (IBD). IBD disease that causes inflammation, which is followed by severe gastrointestinal (GI) symptoms such ulceration of the mucosa and sub mucosa of the colon and the rectum, as well as diarrhea, bleeding, stomach pain, weight loss, anemia, edema, and hemorrhaging (Impellizzeri et al., 2015;(Mandalari et al.,2011). However, an imbalanced immune system response in the intestinal mucosa will appear to induce these illness conditions. The etiology and physiopathology of IBD are still unknown. Tumour necrosis factor (TNF) and interleukin-1 (IL-1) are the two immuno-modulatory cytokines that are most important for enhancing the inflammatory response. These cytokines are present at greater levels in IBD patients (Podolsky et al., 2002, Shanmugam et al., 2020).

MATERIAL AND METHODS

2.1 Animals

Adult male Sprague Dawley Rats body weight (150-250 g) was procured from Central Drug Research Institute (CDRI) Lucknow. They were kept in the departmental animal house, integral university. The animals were housed separately in polypropylene cages for acclimatization at temperature of (23±2℃) and 50-60% relative humidity with a 12 hours light /dark cycle one week before and during the commencement of the experimental animals were kept on standard pellet diet and drinking water ad libitum throughout the study period. The study protocol was approved by institutional Animal committee (IAEC), Faculty of pharmacy, Integral University, Lucknow (Tarique et al., 2019). **(Approval no: IU/IAEC/2129)**.

ISSN: 2519-9889
Impact Factor: 5.9

2.2 Experimental procedure

All the animals were divided into four groups of five after acclimating for a week. The treatment schedule of each group was given in following Table 1.

Table 1.Treatment schedule

S.N.	Groups (n=5)	Dosage, Route of administration and duration
1	Normal Control	10 mg/kg/p. o. body wt. of CMC (1%) once a day for 5 days + and on day five 12 hrs fasted rats were sacrificed.
2	Disease control	10 mg/kg/p, o. (Indomethacin) + 10 mg/kg/ p.o. body wt. of CMC (1%) for 2 consecutive days in 12 hrs fasted rats + and on day third 12 hrs fasted rats were sacrificed.
3	Self-healing	10 mg/kg/p, o.(Indomethacin) for 2 consecutive days in 12 hrs fasted rats + 10 mg/kg/p. o body wt. of CMC (1%) for 5 days + and on day five 12 hrs fasted rats were sacrificed.
4	Test drug	10 mg/kg/p, o. (Indomethacin) for 2 consecutive days in 12 hrs fasted rats + Treated with Biochanin-A (10 mg/kg, p, o.) for 5 days+ + and on day five 12 hrs fasted rats were sacrificed **(Jia B et al., 2023).**

After treatment, rats were euthanized under anesthesia, stomach and colon was removed (Arab HH et al., 2014). The stomach was opened along the greater curvature, gently cleaned with distilled water, and checked for ulcer spots (Vogel et al., 2002). After that colon specimens were cleaned and weighed and weight /length ratio was calculated for the rat. For histo-pathological analysis, a small section of the stomach and colon were removed, and the remaining part of the stomach was immediately submerged in an alcian blue solution to measure the thickness of the mucus wall. (Corne et al., 1974).

Estimation in gastric tissue

2.3.1 Ulcer index

The mucosa was examined under a 10X microscope after the stomach was removed along its largest curve, opened, cleaned with saline, and pinned to a cork plate. In the rat, the upper two fifths of the stomach form the rumen with squamous epithelium and have few defences against the corrosive action of gastric juice.

The defensive mechanisms are better in the mucosa of the middle two fifths of the stomach than in the lowest region, which forms the antrum, below a limiting ridge in the glandular portion of the stomach. Therefore, the rumen and antrum were the primary sites of lesions. The prevalence of ulcers was noted, and the following scores were used to indicate their severity. The number of ulcers was counted. Scoring of ulcers was made. Normal coloration was assigned

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Impact Factor: 5.9

0 = No Ulcer, 1= Superficial ulcer, 2= Deep ulcer and 3=Perforation ulcer (Idris et al., 2023).

An ulcer index UI is calculated:

$U1=U_N+Us+Up\times10-1$

U_N=Average of number of Ulcer per animal

Us=Average of severity score

Up=Percentage of animals with ulcers

Ulcer index of treated animals are compared with self-healing.

2.4 Gastric wall mucus (Barrier mucus determination)

The amount of stomach wall mucus was measured using the technique reported by Corne et al., 1974. Alcian blue dye, which only colors the barrier mucus does not enter the mucosal tissue, was used in this approach. The dye combination with barrier mucus can be broken down by immersion in a standard MgCl2 solution; the optical density of the resultant blue solution was measured at 605 nm.

2.4.1 Procedure

After being opened along their greater curvature, the stomach's glandular portions were taken out and weighed. Each was then immediately transferred to 10 ml of 0.1% w/v alien blue solution, which was in a 0.16 M sucrose solution that had been buffered with 0.05 M sodium acetate and had its pH adjusted to 5.8 using HCI.

Excess dye was removed following a two-hour immersion by rinsing twice in quick succession for 15 and 45 minutes each with 10 ml of 0.25M sucrose. With 10 ml of 0.5 M magnesium chloride, dye complex including stomach wall mucus was extracted over the course of two hours by shaking sporadically for one minute at intervals of 30 minutes.

After rapidly shaking the resultant blue solution with an equivalent volume of diethyl ether, the emulsion was centrifuged at 3000 rpm for 10 minutes, and the absorbance of the aqueous layer against a standard magnesium chloride solution served as a blank was measured at 580 nm. The amount of alien blue collected from was then determined per gram of net glandular tissue (Jafri MA et al., 2001).

2.5 Evaluation of changes in gross anatomical structure of stomach

I Normal control group: A normal group displaying a typical appearance.

II Disease control group: Deep ulcer and severe lesion on indomethacin-induced ulcer.

III Self-Healing group: Self-healing groups show few lesions and superficial ulcers have slightly less as compared to Group II.

IV Test drug group: Biochanin-A plus indomethacin treatment with no ulcer and redness are seen.

2.6 Evaluation of changes in gross anatomical structure of colon

I Normal control group: Mucosal layer appears normal without any redness.

II Disease control: Lesions are prominent as compared to normal colon.

III Self-healing group: Lesions are fewer and superficial as compared to disease control groups.

ISSN: 2519-9889
Impact Factor: 5.9

IV Test drug group: Biochanin -A no disruption is appearing and mucosal lining appears normal in its architecture**.**

2.7 Histopathological studies

The colon and stomach were separated, fixed in paraffin, and stained with hematoxylin and eosin after being placed in a 10% neutral formalin solution. The histology slides were examined under the light microscope.

2.8 Statistical analysis

The analysis was expressed as mean ± standard error of mean (S.E.M). Analysis of Variance (ANOVA) was used to analyse the outcome, and then the Dennett test (Ahmad A et al., 2018).

2.9 RESULT

Effects of Biochanin- A on various ulcer-specific factors

Table 3 shows how Biochanin-A affects various ulcer-specific factors.

2.9.1 Effect of Biochanin -A on ulcer index

The mean ulcer index was significantly (P<0.01) increased in the disease control group of rats (i . e. Indomethacin treated = group II) when compared to normal control group of rats (1% CMC treated = group I). The mean ulcer index significantly (P <0.01) decreased in the Biochanin-A (10 mg/kg b.w t.) treated group (group IV) when compared to the self -healing group (group-III) **(Anbarasi et al., 2006, Nigam et al., 2008).**

Table 3. Effects of Biochanin- A on different variable ulcer index, gastric wall mucus and weight/length ratio of colon parameter

Groups	Ulcer index(UI)	Gastric wall mucus µg/g of stomach	Weight /length ratio
Normal control	0.00±0.0	2.1±0.05	0.0818±0.00086
Disease control	14.546±0.2650**	0.8±0.083**	0.0307±0.0041**
Self-healing	14.04± 0.1300	1.515±0.097	0.03075±0.0017
Test drug	6.5±0.288##	2.02±0.07##	0.073±0.00091##

All values were presented as Mean SD, with n equal to 5. The comparisons were done using ANOVA, and the Dunnett's "t" test followed. ##P 0.01 versus the self-healing groups (group III) and **P 0.01 versus the normal control group (group 1), respectively **(Tahir et al., 2022).**

2.9.2 Effect of Biochanin- A on gastric wall mucus

The average thickness of the stomach wall mucus was determined using the Alcian Blue calibration curve. Rats in disease control groups (group II rats) had considerably (P 0.01) less mean stomach wall mucus thickness than normal control healthy rats (group I rats) (Table 4 & Fig 6).

ISSN: 2519-9889
Impact Factor: 5.9

The group's mean stomach wall mucus thickness considerably (P 0.01) increased after receiving Biochanin-A (10 mg/kg/b.w. t. p. o.) compared to the rats in the self-healing group (i.e., group third) **(Anbarasi et al., 2006**, **Sharma et al., 2011).**

2.9.3 Effect of Biochanin -A on weight /length ratio of colon

When compared to the normal control healthy rats (i.e., group I rats), the disease control group rats (i.e., group II rats) had a significantly (<P0.01) decreased mean weight/length ratio of the colon (Table 4 & fig 2). The weight/length ratio of the colon considerably (P <0.01) increased in the group (i.e. group IV) after receiving Biochanin-A (10 mg/kg/b.wt. p. o.), compared to the rats in the self-healing group (i.e. group 3rd) **(Anbarasi et al., 2006**, **Sharma et al., 2011).**

2.9.4 Effect of Biochanin -A on gross anatomical structure of stomach

Fig. [I] demonstrates how Biochanin-A affects the gross anatomy and structure of the stomach.

I Normal Control group-I: Normal group showed its normal appearance.

II Disease control group-II: Deep ulceration and severe lesions were visible in the indomethacin-induced ulcer.

III Self-healing group-III: Few lesions and superficial ulceration were visible in indomethacin treatment.

IV Biochanin-A IV Indomethacin-induced submucosal inflammation, histological gastric mucosal injury, and macroscopic stomach mucosal injury were all lessened by biochanin (Fig 3).

2.9.5 Effect of Biochanin -A on gross anatomical structure of colon.

In the normal control group the mucosal layer appears normal without any redness. In disease control lesions were prominent as compared to normal colon. In self-healing control groups lesions were fewer and superficial as compared to disease control groups. In a drug treated group (i.e.) Biochanin-A treated no disruption is appearing and mucosal lining appears normal in its architecture **(Fig 4).**

2.9.6 Effect of Biochanin-A on histopathological structure of stomach

I Normal control: Mucosal glands were seen compactly arranged and showed a typical appearance.

II Disease control group: There were stomach ulcers caused by indomethacin that left behind linear hemorrhagic erosions, edoema, and severe inflammation.

III Self-healing group: On occasion present superficial ulcers, mucosal erosion, and moderate inflammation, all of which were significantly less severe than in the disease control group.

IV Test group: Treatment group mucosal lining were smooth with little disruption and minute ulceration in mucosal layer and little edema in sub-mucosal layer **(Fig 7).**

ISSN: 2519-9889
Impact Factor: 5.9

2.9.7 Effect of Biochanin-A on histopathological structure of colon

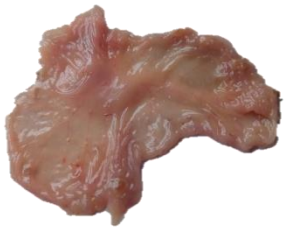
In the normal control group: mucosal layer appeared normal without any redness.
In the disease control group: lesion was prominent as compared to normal colon.
In self-healing group: Lesions are fewer and superficial as compared to disease control groups.
In treatment group: Mucosal lining were smooth with little disruption and minute edema in sub-mucosal layer

2.9.8 Figure 2. Effect of Biochanin-A on weight/length ratio of colon

When n equal to 5, all values were given as Mean SD. The comparisons were done using ANOVA, and the Dunnett's "t" test followed. ** indicates P< 0.01 when compared to the normal control group (group 1) and ## indicates P< 0.01 when compared to the self-healing group (group III).

Normal control

Disease control



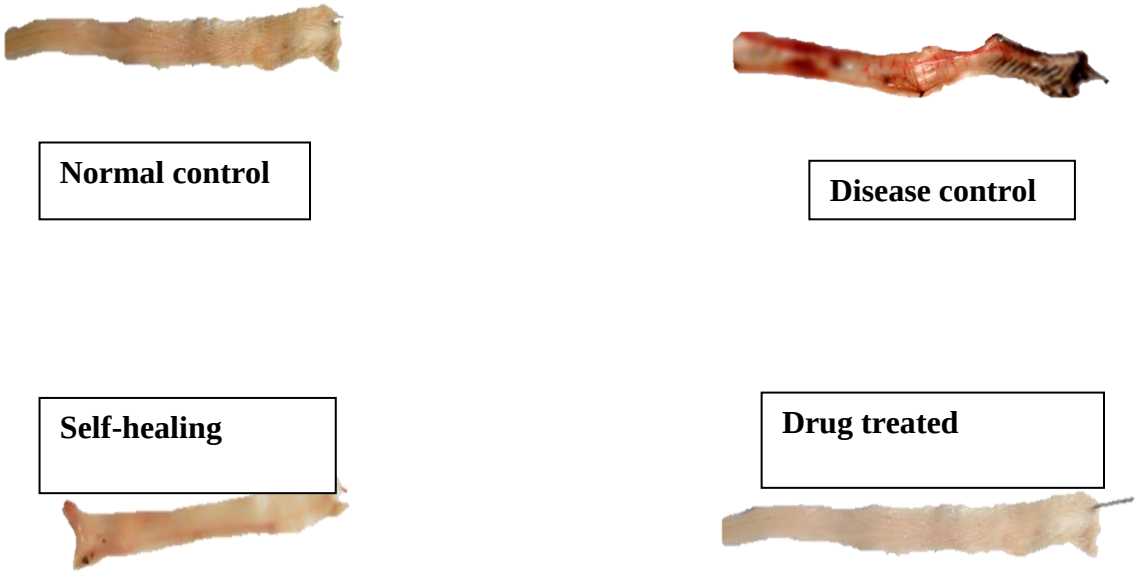
Self-healing

Test drug

2.9.9 Figure 3. Gross anatomical structure of the stomach

I In Normal control group: Normal group showed its normal appearance.
II In Disease control group: Deep ulcer and severe lesion were visible in the indomethacin-induced ulcer.
III In Self-Healing group: When compared to Group II, the self-healing group had fewer superficial ulcers and lesions overall.
IV In drug treated group IV: Redness and no ulcer were observed after treatment with Biochanin-A and Indomethacin.

ISSN: 2519-9889
Impact Factor: 5.9



2.9.10 Figure 4. Gross anatomical structure of a Colon

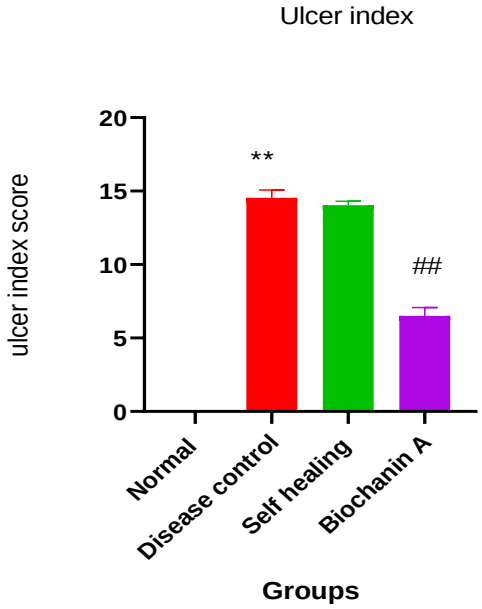
Normal control group I: Mucosal layer appeared normal without any redness.

In **Disease control II** lesions are prominent as compared to normal colon.

In **self-healing control group III** lesions were fewer and superficial as compared to disease control groups.

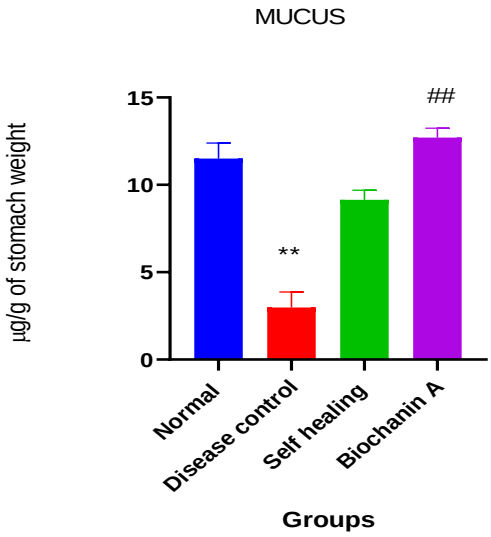
In **drug treated group IV Biochanin-A** no disruption was appearing and mucosal lining appeared normal in its architecture**.**

ISSN: 2519-9889
Impact Factor: 5.9



2.9.11 Figure 5. Effect of Biochanin-A on gastric ulcer

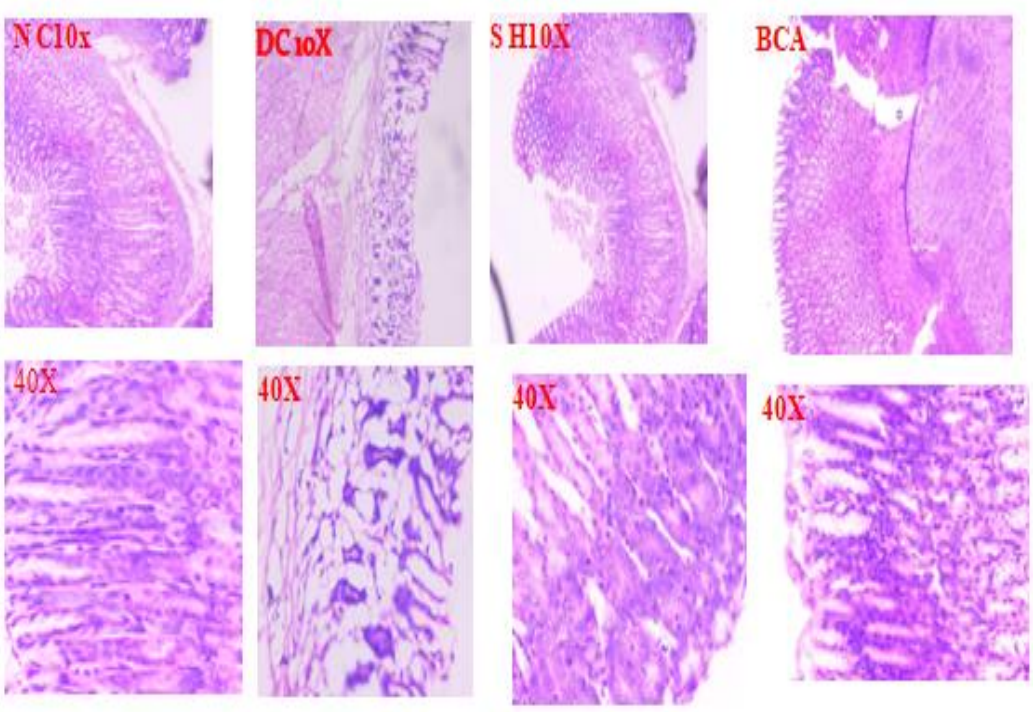
All values were expressed as Mean ± SD, (n =5). The comparisons were done by ANOVA followed by Dunnett's "t' test. ** indicates P< 0.01 as compared to normal control group (i.e. group-1), ## P< 0.01 as compared to Self- healing group (i.e. group-III).



2.9.12 Figure 6. Effect of Biochanin A on gastric wall mucus

All values were presented as Mean SD, with n equal to 5. ANOVA was used for the comparisons, then the Dunnett's "t" test. ## P< 0.01 as compared to the Self-healing group (i.e. group-III), and ** denotes P< 0.01 as compared to the normal control group (i.e. group-1).

ISSN: 2519-9889
Impact Factor: 5.9



Abbreviation NC=Normal control, DC=Disease control, SH=Self-Healing, BCA=Biochanin A

2.9.13 Figure 7 Histopathological structure of stomach

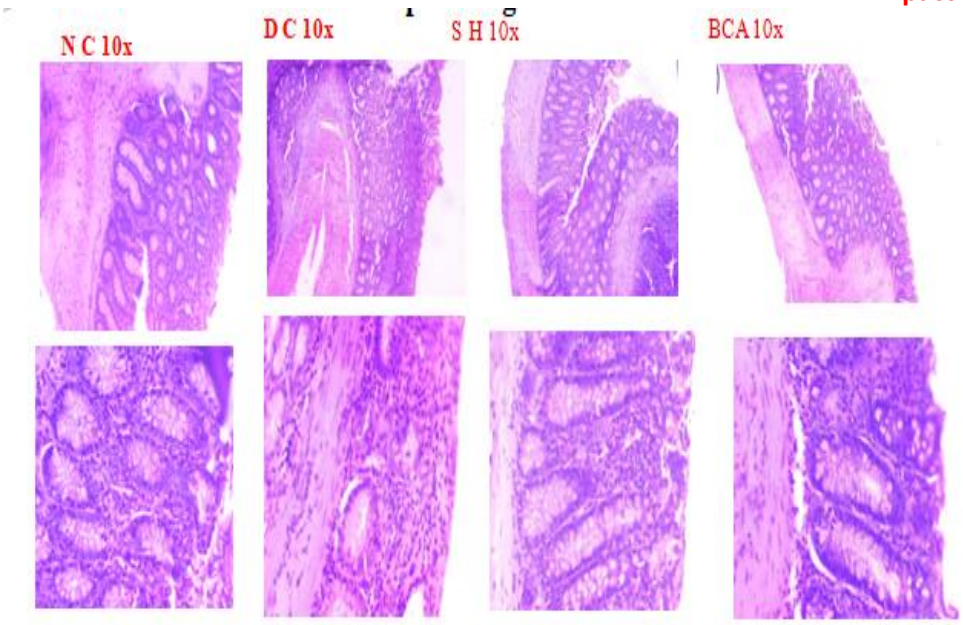
I Normal control I: Mucosal glands were seen compactly arranged showing its normal appearance.

II Disease control group: A number of stomach ulcers caused by indomethacin that left behind linear hemorrhagic erosions, edema, and severe inflammation.

III Self-healing group III: Moderate inflammation, which was much less than the disease control group, with occasional superficial ulceration and mucosal erosion.

IV Test group IV: Treatment group mucosal lining were smooth with little disruption and minute ulceration in mucosal layer and little edema in sub-mucosal layer.

ISSN: 2519-9889
Impact Factor: 5.9



Abbreviation NC=Normal control, DC=Disease control, SH=Self-Healing, BCA=Biochanin A

2.9.14 Figure 8 Histo-pathological structure of colon

In normal **control group:** Mucosal layer appeared normal without any redness.
In disease **control group:** Lesion was prominent as compared to normal colon.
In **self-healing group:** Lesions were fewer and superficial as compared to disease control groups**.**
In **treatment group**: Mucosal lining were smooth with little disruption and minute edema in sub-mucosal layer**.**

DISCUSSION

A combination of their analgesic and anti-inflammatory effects, NSAIDs are still frequently used throughout the world. In this field, indomethacin is frequently used. The progression of the indomethacin-induced gastric mucosal lesions is significantly influenced by the production of free radicals from oxygen and lipid peroxide in particularly. However, it is widely known that indomethacin can lead to severe and extensive stomach mucosal erosions and ulcers. Use of indomethacin is severely constrained by these adverse effects (Cashing et al., 1997).
Non-steroidal anti-inflammatory drugs (NSAIDs) are among the most significant medications that cause PUD. It has been demonstrated that NSAID use is associated with an increase in mucosal damage, erosions, duodenal bleeding, and gastric bleeding. These effects are mostly brought on by the suppression of cyclo-oxygenase-1, the formation of thromboxane-A2, and reactive oxygen species (ROS). For the treatment and management of PUD, a wide range of medications including prostaglandin analogues, proton pump inhibitors, cytoprotective medicines, and histamine receptor antagonists are available. But when used repeatedly, the

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Impact Factor: 5.9

majority of them cause a variety of harmful drug reactions and can even alter the body's biochemical pathways (Khushtar et al., 2016).

The pathophysiology of gastric ulceration is multifactorial. Hypersecretion of pepsin and hydrochloric acid can lead to ulcers by disrupting the balance of gastric luminal components and weakening the protective function of the stomach mucosal barrier. Examples of mucosal protection include mucus, bicarbonate secretion, mucus blood flow, and epithelial cell defence. When acid and pepsin pass through the mucosal barrier, histamine is produced. As long as this vicious cycle continues, erosion will occur, leading to the development of the ulcer. Parietal cells will release more acid when histamine is present (Malfertheiner et al., 2009).

However, mixed glands, which contain both serous and mucous cells, can also generate it, mucous gland cells typically do so. The epithelial cells that surround the respiratory, digestive, and urogenital systems, as well as the visual and auditory system structures, are all shielded from pathogenic fungus, bacteria, and viruses by mucus. The digestive system produces most of the body's mucus (Tripathi et al., 2003).

Smoking inhibits mucosa blood flow and healing whereas prolonged NSAID use decreases mucosal prostaglandin synthesis. Alcohol has not been proven to be the direct cause of peptic ulcers, but cirrhosis caused by alcohol is associated with a higher prevalence of peptic ulcers. Corticosteroids promote the growth of ulcers when administered often and in large amounts. Some people who have duodenal ulcers have very rapid stomach emptying, which exposes the duodenal mucosa to an excessive amount of acid. Duodenal ulcers are more prone to form in people with alcoholic cirrhosis, chronic obstructive pulmonary disease, chronic renal failure, and hyperparathyroidism. It is believed that one of the most important defences against stomach damage is the creation of mucus. (Cotran et al., 2000).

Studies haven't confirmed stress as a cause of inflammatory bowel diseases (IBD). However, many people who have forms of IBD have reported that stress worsens their symptoms. Moderate exercise has been shown to reduce stress and improve the immune system, but several studies have also found that getting too much exercise or exercising at a high intensity can trigger or worsen symptoms.

The latter generally occurs before the bowels act or when there is a lot of flatulence; local tenderness on deep pressure over the colon is varied. Abdominal discomfort is more common than genuine pain. The value of a sigmoido-scopic examination cannot be overstated at the current time; even though it only examines a piece of the large intestine, that region typically reveals ulceration if it is present. While appetite and digestion are largely maintained, diarrhoea and toxaemia cause weight loss and weakness. Relapses are common in these cases. The sigmoidoscope is the obvious tool for diagnosing the chronic instances of colon cancer, tuberculosis, and follicular ulceration (Feinman et ., al 2010).

An interesting bioactive substance called Biochanin-A is present in numerous conventionally used medicinal herbs. It has antispasmodic and anti-inflammatory properties. A study was done in vivo to see how well Biochanin-A protected against colitis and stomach ulcers brought on by indomethacin. Red clover contains the naturally occurring food isoflavone Biochanin-A (BCA), which is also found in various herbal dietary supplements. BCA is transformed into its conjugate genistein during metabolism. The medicinal evaluation of

ISSN: 2519-9889
Impact Factor: 5.9

BCA has recently increased due to the alleged favourable features it might offer to cure various human ailments. BCA in particular has actions that are anti-inflammatory, anti-carcinogenic, anti-cancer, and have antioxidant activity.

Inflammation, edoema, and the loss of epithelial cells are the characteristics of Indomethacin-induced damage, according to prior research' histological analysis of the stomach tissue. The current study's findings demonstrated the defensive effectiveness against Indomethacin-induced stomach injury after the drug's administration effect on banding. The circular muscles that guide mucosal density to the ridges of mucosal layers were noticeably reduced by indomethacin, which led to necrosis and ulceration.

The present study was designed in recognition of the opportunities in the investigation of medications described in the conventional system. Investigate the effects of Biochanin-A on stomach ulcer and colitis produced by indomethacin in experimental rats was the problem stated for the current research project. Other biological effects of BCA include anti-proliferative, anti-inflammatory, dopaminergic neuron protection, stimulation of osteoblastic differentiation, and melanogenesis inhibition. BCA appears to be a possibility for the advantages to human health (Hajrezaieet al., 2015).

Indomethacin-induced stomach ulcer model can be utilised to create severe ulceration in lab animals, say Saheed et al., (2014). Indomethacin harms mucosal tissues by affecting prostaglandin synthesis, acid secretion, and back diffusion of H+ ions (Ran et al., 2000). The oral ingestion of indomethacin causes a rapid inhibition of mucosal prostaglandin synthesis. This is related to how quickly the mucosa absorbs these drugs. Thus, in our investigation, rats were given indomethacin to induce severe ulceration.

The results of this study demonstrated that Group I, the normal control group, which was fed simply a basal diet and CMC, produced normal levels of mean ulcer index and mean stomach wall mucus thickness. It was observed that Indomethacin (10 mg/kg, p. o)) treated rats (i. e. group II) showed significant ($P<0.01$) increased levels of mean ulcer index and significant decreased in mean gastric wall mucus thickness as compared to the normal control rats (i.e. group I). When compared to the self-healing group and the indomethacin-treated group, treatment with Biochanin-A (10 mg/kg, p.o.) results in a substantial decrease in mean ulcer index and a significant rise in stomach mucus content (Devaraj et al., 2007, Devaraj et al., 2007).

Various scoring systems have been developed which quantify gastric ulceration by calculating ulcer index. Although the number of ulcers has been given the highest priority in all scoring systems when determining the severity of the condition, other factors such as the ratio between the area of the gastric mucosa and the area of ulceration or perforation, as well as the frequency distribution of ulcer size, should not be ignored. Additionally, the extent of the ulcer index may be a good indicator of the degree of stress present or the ulcero-genic potential of certain medications (Parmar et al., 1993, Ganguly et al., 1969).

When compared to rats treated with indomethacin (group II rats) in the current study, test drug-treated rats (group IV) showed a significantly ($P <0.01$) lower mean ulcer index. The results of Hussain et al., 2015 and Khustar et al., 2016, who both showed reductions in ulcer index following the administration of an aqueous extract of Crica papaya L. seed to rats that had received an induction dose of indomethacin, are consistent with a decrease in ulcer index.

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The integrity of the stomach mucosa depends on the proper balance of HCI, pepsin, protective substances like mucus and HCO3, secretion, prostaglandins, mucosal blood flow, and nitric oxide. The primary therapy recommendations consequently center on reducing acid secretion and boosting the development of compounds that shield the stomach mucosa, preventing epithelial damage. The treatment with Biochanin-A (10 mg/kg/p. o.) group IV rats significantly ($P<0.01$) increased mean stomach wall mucus thickness in comparison to the self-healing (i.e. group III rats) and indomethacin treated groups. Our findings corroborated those of Shoaib et al., 2014), who discovered that in rats with gastric ulcers brought on by indomethacin, indomehacin and pylorus ligation, the hydromethanolic extract of Andrographitis paniculata enhanced the thickness of stomach wall mucus (Klein et al., 2010).
A positive change in the weight/length ratio of the colon has been linked to a reduction in the symptoms of colitis, according to research by Vijayabharathi et al., 2018. In our investigation, administration of Biochanin-A improved the weight-to-length ratio of the colon, showing that indomethacin-induced colitis had subsided (Panéset al., 2007).

2.11 Conclusion

As shown by the strengthening of the gastric mucosa, reduction in ulcer index, and increase in gastric wall mucus, our results suggested that therapy with Biochanin-A had gastro-protective effects in Indomethacin-induced gastric ulcer in experimental rats (Khushtar et al., 2016).
Biochanin A also has been shown to useful in preserving the intestinal mucosa by increasing the weight/ length ratio. The anatomical changes to the stomach and colon brought on by indomethacin exposure were fixed in rats receiving Biochanin-A therapy.
Biochanin-A is an isoflavone and thought to have anti-inflammatory and antioxidant properties. These characteristics may be responsible for Biochanin-A's success in treating colitis and stomach ulcers.

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CONFLICT OF INTEREST
Authors declared for none conflict of interest.

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