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EFFECT OF ELLAGIC ACID AND ITS COMBINATION WITH MESALAZINE ON EXPERIMENTALLY INDUCED INFLAMMATORY BOWEL DISEASE

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ABSTRACT

Introduction

IBD includes a scope of digestive pathologies, the most well-known of which are UC and CD. Both UC and CD, when present in the colon, produce a comparable side effect profile which can incorporate looseness of the bowels, rectal dying, and weight loss.

The Mesalazine is an anti-inflammatory agent structurally related to the salicylates and non-steroidal anti- inflammatory drugs like acetylsalicylic acid, which is active in inflammatory bowel disease. Ellagic acid is a natural anti-oxidant phenol found in numerous fruits in particular abundant amount pomegranate. In pomegranates, there are several therapeutic compounds but Ellagic acid is the most active and abundant. Ellagic acid is also present in vegetables the anti-proliferative and anti-oxidant properties of Ellagic acid have prompted research into its potential health benefit. We aimed to

investigation of Ellagic acid alone and its combination with Mesalazine on experimentally induced IBD in mice.

Materials and Methods:

Colitis severity and inflammation-associated damage can be assessed by examining stool consistency and bleeding, In the acute UC model, female Balb/C mice were treated with DSS (5%) for seven days while concomitantly receiving a combination of dietary supplement of ellagic acid (2%) and anti-inflammatory agent Mesalazine in the IBD model,

Result:

Ellagic acid combination with Mesalazine significantly improved changes in the length of the colon and inhibited the progression of the disease, reducing intestinal inflammation and decreasing histological scores in DSS-induced colitis in mice.

Conclusion:

The result shows that treatment with Ellagic Acid and Mesalazine individually is able to improve the inflammatory conditions by suppressing the various inflammatory mediators.

Keywords: Inflammatory Bowel Disease, Ellagic acid, Mesalazine, DSS

INTRODUCTION

IBD is caused by a combination of genetic and environmental factors that influence immune responses. The two most common inflammatory bowel diseases are UC and CD. Crohn's disease and ulcerative colitis are both chronic IBD that cause digestive problems and inflammation in the GI tract. It is mostly inflammation characteristic **[1]**. Both children and adults suffer from both illnesses and men and women are affected similarly. Despite the similarities in the symptoms of these two conditions, there are some distinctions between CD and UC symptoms **[2]**.

Three doctors—Burrill Crohn, Leon Ginzberg, and Gordon D. Oppenheimer—described Crohn's disease for the first time in 1932. Intestinal tuberculosis was considered to be any disease affecting the small intestine at the time.

Crohn's disease is affects the mouth, anus, and all the layers of intestine. The

mucosal layer of the colon is affected by ulcerative colitis. The rectum and intestine are also affected. Two English physicians, Wilks and Moxon, first identified colitis ulceration in 1875, differentiating this colitis from infectious disease diarrhoea. While it was not designated as a separate disease until 1875, an illness with related symptoms of ulcerative colitis is recorded both before and many years before the civil war **[3]**.

Blood in the stool, extreme pain, and diarrhoea are all signs of ulcerative colitis, while in severe cases of CD, there is often a risk of bleeding. Rectal bleeding is less common in CD, although rectal bleeding is more common in UC. More than half of people with CD have a deficiency in folate and vitamin D, while more than half of people with UC have an iron deficiency **[4]**.

Both children and adults are affected. UC is thought to affect 2.6 million people in Europe and 1.2 million in North America. Around a fifth of these patients are diagnosed before they reach the age of 18. IBD is commonly diagnosed in puberty, and about 25% of IBD patients are under the age of 20.

UC is more common than Crohn's disease around the world. Both diseases are more prevalent in the developed world, especially in North America and Western Europe, though their prevalence is growing in Asia **[5]**. Overall, there are 1.2 to 20.3 cases per 100,000 people per year, with an incidence of 7.6 to 245 cases per 100,000 people per year.

Irritable bowel syndrome is yet to be pinpointed as a cause. The immune system of the body is triggered to develop an inflammatory response in the intestinal tract by some agent or a combination of agents, such as bacteria, viruses, and antigens. IBD is thought to be caused by a combination of hereditary, genetic, and environmental factors, according to recent research **[6, 7]**. It's also probable that the bodies own tissue is to blame autoimmune response. Whatever causes it the reaction continues without control and damages the intestinal wall, leading to diarrhea and abdominal pain.

Preclinical animal models of IBD are crucial for gaining a better understanding of the disease's pathogenesis. IBD is a chronic inflammatory disorder with two main classifications:

UC and CD **[8, 9]**.

IBD is characterized by diarrhoea that is accompanied by blood, mucus, and pus in the stool.

UC is characterized by inflammation of the colon mucosa, loss of mucus-forming goblet cells, crypt irregularity, and crypt abscesses, as well as neutrophils surrounding local blood vessels, plasma cell infiltration, and lymphoid aggregates in the lamina propria **[10, 11]**. Inflammatory lesions are most commonly found in the ileum and colon in Crohn's disease. With extensive granuloma formations, these lesions are transmural.

The Mesalazine is an anti-inflammatory agent structurally related to the salicylates and non-steroidal anti-inflammatory drugs like acetylsalicylic acid, which is active in inflammatory bowel disease. It is considered to be the active moiety of sulphasalazine. It is used to treat ulcerative colitis and Crohn's disease.

Ellagic acid is a natural anti-oxidant phenol found in numerous fruits in particular pomegranate, persimmon, raspberry, black raspberry, strawberry, peach, plumes, nuts (walnuts, almonds), and wine. In pomegranates, there are several therapeutic compounds but Ellagic acid is the most active and abundant. Ellagic acid is also present in vegetables the anti-proliferative and anti-oxidant properties of Ellagic acid have prompted research into its potential health benefit.

Some people take Ellagic acid by mouth for cancer, mental function, and viral or bacterial infections.

We aimed to investigation of Ellagic acid alone and its combination with Mesalazine on experimentally induced IBD in mice.

MATERIALS AND METHOD

Experimental Animals Healthy mice (Either male or female) weighing 18-30g were used for the study. All experiments and protocols described in the present study were approved by the IAEC of Pharmacology department, Parul institute of pharmacy and research and with permission from CPCSEA.

Animals were procured from Sun pharma Research Center, Ahmedabad. Mice were allowed for acclimatization for seven days on pelleted standard rat food with

water and housed in a group of 3 mice per cage under well-controlled standard conditions of temperature (22±2ºC), humidity (55±5%) and 12hrs light conditions and 12hrs dark condition cycle in animal house.

Induction of IBD by using DSS (Dextran Sodium Sulphate)

Prepare an optimized concentration of DSS in sterile water by weighing DSS powder and mixing until a clear solution is achieved. Administered the 2-3% of DSS orally for 7 days. Measure body weight daily and Collect feces by placing single mice in an empty cage without bedding material for 15 to 30 min. With sterile forceps, collect feces and observe. Body weights may slightly increase during the first 3-4 days and begin to decrease gradually with the initiation of bleeding. On the day of sacrifice, mice should be deprived of food for 4h. Spray 70% ethanol and carefully open the mice by ventral midline incision. Lift the colon with forceps and carefully pull until the cecum is visible. Isolate the colon and cecum by separating them from the small intestine at the ileocecal junction and from the anus at the distal end of rectum. To measure length, straighten but do not stretch the colon. The colon can then be separated from the cecum and wash (5-10 ml syringe with feeding needle using cold PBS to remove feces and blood. Cut colons into pieces depending on need. The piece of colon stored in formalin for Histology study.

Experimental Design

Study was carried out for 28 days. Group-I animal were received normal saline solution orally Group-II received DSS (2%) for 7 days orally. Group-III were received Ellagic acid (25mg/kg, p.o.) Group IV- received Mesalazine (100mg/kg, p.o.) Group V-were received combination of Ellagic and Mesalazine for 21 days on 7th day of the study, IBD was induced with DSS in animals of all groups.

Group	Treatment
Group – I	Normal
Group – II	control
Group – III	Ellagic acid (25 mg/kg)
Group – IV	Mesalazine (100 mg/kg)
Group – V	Combination of Ellagic acid + Mesalazine

On 21st day, the animals were weighed and anaesthetize with ether, and the abdomen will be opened by a middle incision. The colon was removed, free from surrounding tissue, rinse and weight of it was measured. From each group one colon of randomly selected animal was store in the formalin and will be use for the histopathology study.

Evaluation Parameters:-

- Stool consistency
- Gross Rectal Bleeding
- Body Weight
- IL-6
- TNF-α
- Histopathological study

RESULTS

Stool consistency: -

Stool consistency of mice before induction was normal but after period of 7 days induction blood was seen with loose stool after the treatment using by administration of Ellagic acid and Mesalazine the stool consistency was normal **(Table 1)**.

Body Weight of mice it was normal before induction but during induction period 4th and 5th day the weight of animal increase and suddenly decrease due to production of the IBD **(Table 2)**.

Table 1: Effect of mesalamine alone and its combination with ellagic acid on changes in stool consistency on DSS induced IBD rats

Sr. No.	Group No.	Group Name	Stool Consistency					
			Before Induction	0 Day	7 Day	14 Day	21 Day	28 Day
1	Group I	Normal Control	2	2	2	2	2	2
2	Group II	Disease Control	2	0	1	0	2	2
3	Group III	Test I (Mesalamine)	2	0	1	0	2	2
4	Group IV	Test II (Ellagic Acid)	2	0	1	0	2	2
5	Group V	Test III (Mesalazine+ Ellagic Acid)	2	0	1	0	2	2

LEGENDS	Stool Consistency
0	loose
1	loose with blood
2	Normal

Table 2: Effect of mesalamine alone and its combination with ellagic acid on changes in body weight on DSS induced IBD rats

Group	Treatment	Dose	Body Weight (Grams) (Mean ± SEM)
1	Normal Control	Normal Saline	33.5±1.37
2	Disease Control	(DSS 2-5% p. o.)	23.16±1.16*
3	Test I	DSS+ Ellagic acid – (25mg/day p. o)	32.66±1.86
4	Test II	DSS+Mesalazine – (100mg/kg p. o)	33.5±2.07
5	Test III	DSS+ Ellagic acid+ Mesalazine – p. o.)	34.5±1.04

Each data represents the mean ± SEM of 06 rats. P value *p<0.05, **p<0.01, ***p<0.001 compared to Normal group. NC= Normal Control (Group -I), DC= Disease Control, Test-1= DC + Ellagic acid (25mg/kg) (Group – III), Test-1I= DC + Mesalazine (100mg/kg), Test-III= DC + Combination of Ellagic Acid +Mesalazine

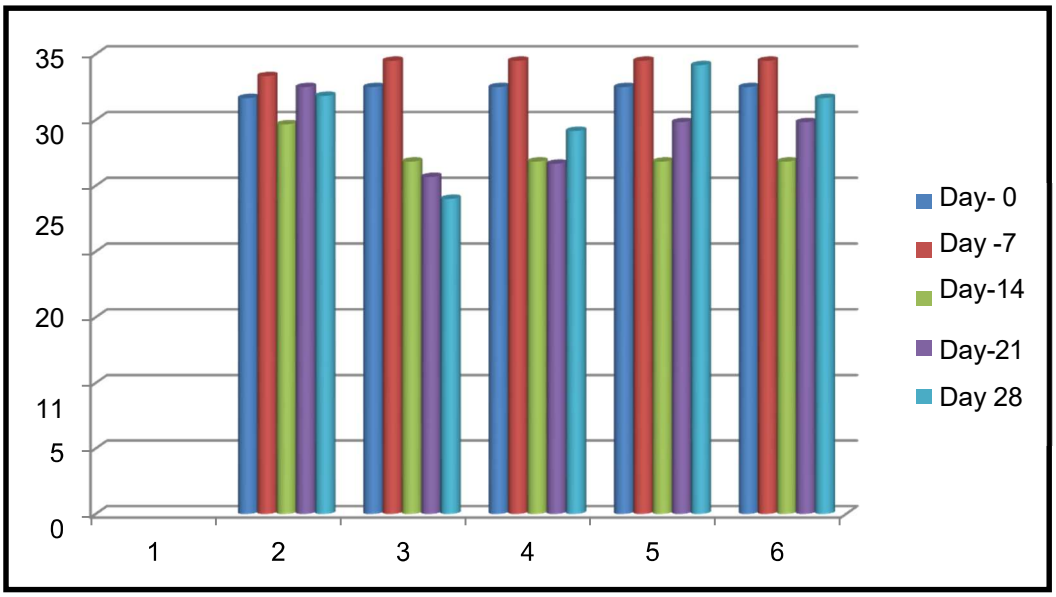

Figure 1: Effect of mesalamine alone and its combination with ellagic acid on changes in body weight on DSS induced IBD rats. NC= Normal Control (Group -I), DC= Disease Control (Group -II), Test-1= DC + Ellagic acid (25mg/kg) (Group – III), Test-2 = DC + Mesalazine (100mg/kg) (Group – IV), Test-3= DC + Combination of Ellagic Acid +Mesalazine (Group – V)

Table 3: Effect of mesalamine alone and its combination with ellagic acid on changes in IL 6 on DSS induced IBD rats

Group	Day-0	Day - 4	Day 7	Day 14	Day 21	Day 28
1	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
2	0.0 ± 0.0	6.7 ± 2.6*	252.5 ± 53.5**	368.9 ± 50.4*	477±54.4*	503±42.4*
3	0.0 ± 0.0	4.2 ± 4.2#	437.5 ± 82.0#	80.2 ± 29.6#	76.3±20.3##	82.3±14.3##
4	0.0 ± 0.0	0.0 ± 0.0	58.1 ± 14.2##	21.5 ± 5.6##	41.0 ± 6.0##	42.0±5.0##
5	0.0 ± 0.0	0.0 ± 0.0	13.4 ± 2.1###	41.0 ± 6.0###	30.1±5.2###	31.7 ± 6.8###

Each data represents the mean ± SEM of 06 rats. P value *p<0.05, **p<0.01, ***p<0.001 compared to Normal group. P value #p<0.05, ##p<0.01, ###p<0.001 compared to Disease group.

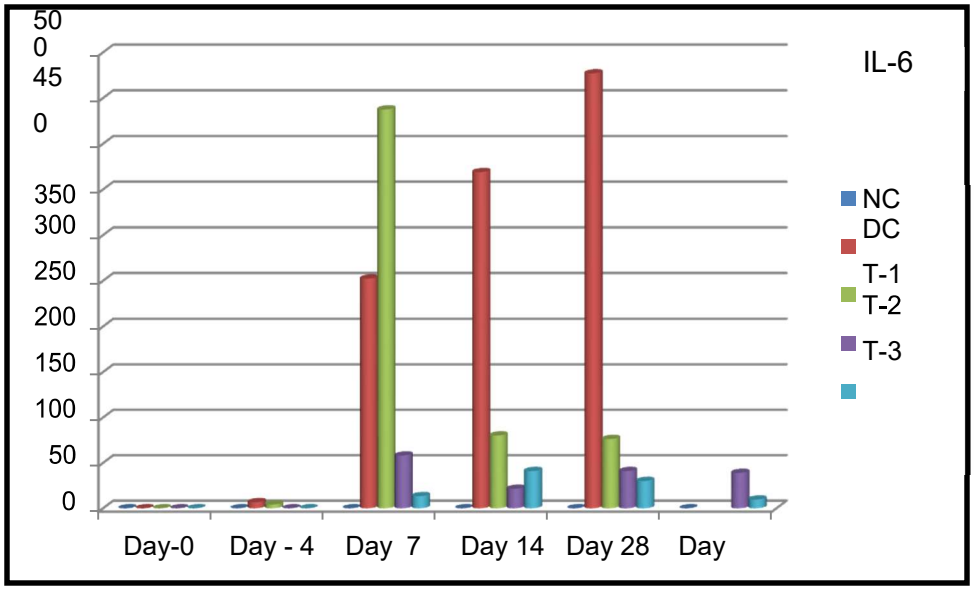

Figure 3: Effect of mesalamine alone and its combination with ellagic acid on changes in IL-6 on DSS induced IBD rats. NC= Normal Control (Group -I), DC= Disease Control (Group -II), T-1=Test-1(Ellagic acid (25mg/kg)) (Group – III), T-2= Test – 2 (Mesalazine (100mg/kg)) (Group – IV), T-3= Test – 3 (Combination of Ellagic Acid +Mesalazine (Group – V)).

Histopathological Study:-

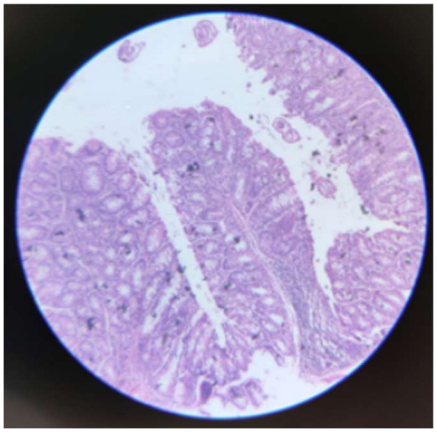

Figure 4: Histological examination of the mice colonic mucosa Group I Normal Control

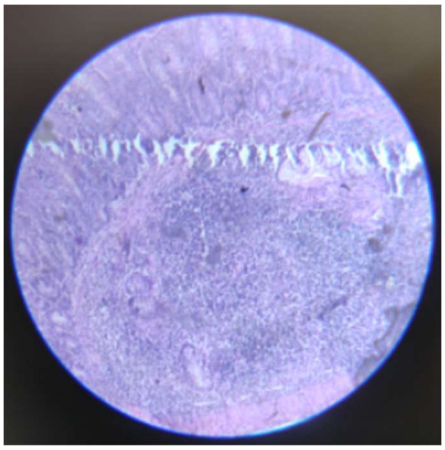

Figure 5: Histological examination of the mice colonic mucosa Group II Disease Control

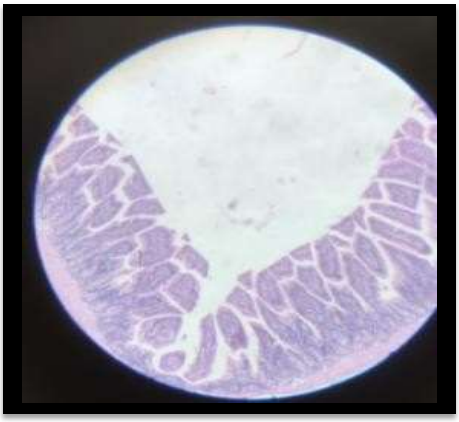

Figure 6: Histological examination of the mice colonic mucosa Group III test-1 Test

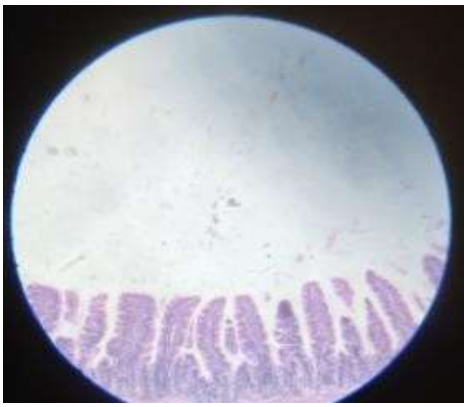

Figure 7: Histological examination of the mice colonic mucosa Group IV 2 Test-2

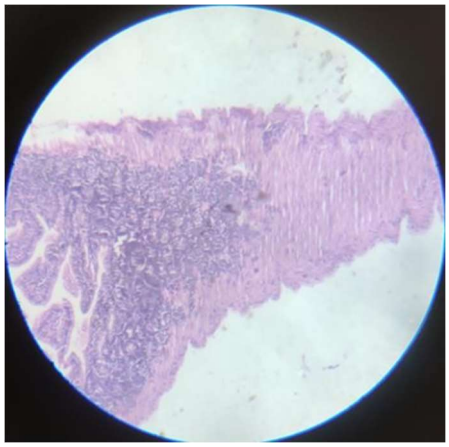

Figure 8: Histological examination of the mice colonic mucosa Group V Test-3

DISCUSSION

The animals of group II were injected by DSS for induction of IBD. After induction period of seven days there was observe rectal bleeding, diarrhoea & loose stool in all the animals. This confirmed the induction of IBD.

During induction period 4th and 5th day the weight of animal increase and suddenly weight decrease due to production of the IBD. After 14 Days of treatment Group-III & Group-IV the weight of animals is normal.in Group-V combination of both drug in this the weight of animal is increases more as compared to other group.

After 28 days Group-III & Group-IV the stool consistency is normal in combination Group-V there is normal stool observed. In histopathological study

Group-II there is Crypt abscesses, granuloma, Necrosis Cryptitis is observed In Group-III Decrease the swelling of colon. Group-IV decrease intestinal inflammation and histological scores. Group-V in this combination of drug there is no observed epithelial degeneration, Necrosis and the lining of the colon is normal there is no lesion is observed.

CONCLUSION

The result shows that treatment with Ellagic Acid and Mesalazine individually is able to improve the inflammatory conditions by suppressing the various inflammatory mediators. But the combination of this Ellagic Acid and Mesalazine produce more significant effect than the individual one. There combination shows more positive effect on histopathology and biochemical estimations than the individual drug effect. So the combined drug treatment of IBD might stand as positive future treatment aspect for the inflammatory conditions of Bowel Disease.

Acknowledgement

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Conflict of Interest

The authors declare no conflict of interest.

Abbreviations

UC: Ulcerative Colitis; CD: Crohn's Disease; DSS: Dextran Sulfate Sodium; IBD: Inflammatory Bowel Disease; IAEC: Institutional Animal Ethics Committee; CPCSEA: Committee for the purpose of control and supervision of experiments on animals

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