

## A comparative study of the effect of Asacol and the ethanolic extracts of *Matricaria chamomilla* and *Terminalia chebula* on induced ulcerative colitis in rabbits

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### Abstract:

Twenty four local rabbits were used in this study separated into four groups. Ulcerative colitis (UC) was induced in all rabbits by rectal administration of 5% glacial acetic acid-30% ethanol (GAA 5%). Animals in the control group were orally (p.o) administered with distilled water, group 2 was administered with Asacol at a dose of 800 mg/kg BW/day p.o., where as group 3 was given ethanolic extract of Chamomile at a dose of 500 mg/kg BW/day p.o, and finally; the rabbits of group four were received 1000 mg/kg BW/day p.o of ethanolic extract of Haritaki.

Each of the above agents was administered two days prior to induction of UC (as a single daily dose), the day of induction, and a dose 22 hours post-induction (i.e., 2 hours prior to sacrificing of the animal). The effects were observed as changes in the serum electrolytes (sodium and potassium), changes in haematological aspects including; Total white blood cell count (total WBC), lymphocytes count, Red blood cell count (RBC), Blood haemoglobin (HGB), and Packed cell volume (PCV%). Besides the examination of gross and histopathological changes of the colon segments for all the tested rabbits.

In conclusion, GAA 5%-induced colitis in rabbits is preferred for testing the anti-inflammatory and anti-ulcer effectiveness of new therapeutic modalities. Asacol showed high potent anti-inflammatory and protective effect against ulceration and inflammation of colon. As well as, ethanolic extracts of both Chamomile and Haritaki have an accepted prophylactic activity against GAA 5%-induced colitis in rabbits through correction of the changed electrolytes, haematological parameters and histopathological signs.

دراسة مقارنة حول تأثير عقار الأساكول والمستخلصات الايثانولية لنباتي البابونج  
والأهليلج على التهاب القولون التقرحي والمحدث تجريبيا في الأرانب

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**الخلاصة:**

تضمنت هذه الدراسة استخدام اربع وعشرون ارنبا محليا مقسمة الى اربع مجاميع. وقد تم استحداث التهاب القولون التقرحي في جميع هذه الأرناب بواسطة الحقن المستقيمي لمحلول يتكون من حامض الخليك الثلجي بتركيز 5% والايثانول بتركيز 30%. حقنت حيوانات مجموعة السيطرة بالماء المقطر وذلك عن طريق الفم، في حين حقنت حيوانات المجموعة الثانية بعقار الأساكول وبجرعة 800 ملغم/كغم من وزن الجسم/يوم فمويا، بينما اعطيت حيوانات المجموعة الثالثة المستخلص الايثانولي لنبات البابونج بجرعة 500 ملغم/كغم من وزن الجسم/يوم فمويا، وأخيرا فان حيوانات المجموعة الرابعة تناولت جرع يومية من المستخلص الايثانولي لنبات الاهليلج بواقع 1000 ملغم/كغم من وزن الجسم/يوم وعن طريق الفم. ان كل واحدة من المواد المذكورة في اعلاه تم حقنها قبل يومين من استحداث التهاب القولون التقرحي (على شكل جرعة مفردة يوميا)، ثم في يوم استحداث المرض، تلتها جرعة اخيرة بعد 22 ساعة من حقن محلول استحداث المرض (أي قبل ساعتين من قتل الحيوانات). كما ان تاثيرات هذه المواد تم ملاحظتها من خلال التغيرات الحاصلة في قيم شوارد المصل (الصيديوم والپوتاسيوم)، وكذلك تغيرات الصورة الدموية التي شملت التعداد الكلي لكريات الدم البيضاء، تعداد الخلايا اللمفية، تعداد كريات الدم الحمراء، هيموغلوبين الدم، والنسبة المئوية لحجم خلايا الدم المرصوص، الى جانب فحص التغيرات العيانية والنسجية المرضية لمقاطع القولون المأخوذة من جميع الحيوانات المختبرة. وكأستنتاج نهائي، فان حامض الخليك الثلجي بتركيز 5% والمستخدم في استحداث التهاب القولون التقرحي في الأرناب يمكن أن يفضل في اختبار الفعاليات المضادة للتقرحات والالتهابات الناتجة من استخدام أنظمة علاجية جديدة. لقد أظهر عقار الأساكول قوة عالية كمضاد للالتهابات وتأثير واقى ضد تقرح والتهاب القولون هذا بالاضافة الى امتلاك المستخلصات الايثانولية لنباتي البابونج والاهليلج فعالية واقية يمكن قبولها ضد التهاب القولون التقرحي والمحدث تجريبييا من خلال تصحيح التغيرات الحاصلة في الشوارد والدلائل الدموية والعلامات النسيجية المرضية.

**Introduction:**

Ulcerative colitis (UC), is a chronic and relapsing inflammatory disease caused by the inflammation and sores in the lining of large intestine (1, 2). Conventional therapy for UC includes sulfasalazine and other 5-aminosalicylic acid (5-ASA) type of compounds such as mesalazine (Asacol)<sup>®</sup> (3), and in more persistent and/or severe cases, oral, rectal and parenteral corticosteroids and immunosuppressants are administered (4). All of these have significant toxicities and are partly or completely ineffective in significant numbers of cases (5). The use of medicinal plants or their active components with anti-ulcer

activity has become an increasingly attractive approach for the treatment of UC. *Matricaria Chamomilla* or *Matricaria recutita* or German chamomile, also spelled chamomile, is one of the most widely used and well-documented medicinal plants. It has been included in the pharmacopoeia of 26 countries. It has been shown that amino acids, polysaccharides, fatty acids, essential oils, mineral elements, flavonoids, and other phenolic compounds are the main constituents of *Matricaria chamomilla* (6, 7). Preparations (e.g. ointments, inhalations, tinctures, teas) of chamomile are using in modern medicine primarily for their

spasmolytic, antiphlogistic, anti-inflammatory, and antibacterial properties (8, 9, 10). One of the chamomile's main roles is its use as a multipurpose digestive aid to treat gastrointestinal disturbances including flatulence, indigestion, diarrhea, anorexia, motion sickness, nausea, and vomiting. Chamomile is thought to heal ulcers and acts as an herbal bitter to stimulate the liver. It has been shown that the extracts from this plant, singly or combined with other plants have antiulcerogenic activity (11). *Terminalia chebula* (Haritaki) is another anti-ulcer agent containing active ingredient including; glycosides such as triterpenes arjunglucoside I, arjungenin, and chebulosides I and II also phenolic compounds such as ellagic acid, 2,4-chebulyl-D-glucopyranose, chebulinic acid, gallic acid, ethyl gallate, punicalagin, terflavin A, terchebin, luteolin, and tannic acid. *Terminalia chebula* preparations used in many conditions like digestive disorders, irregular fevers, flatulence, and constipation (12). It is the best bowel cleanser and can be helpful emptying the stomach in a safer way. *Terminalia cheubla* is basically astringent, mild and safe purgative, stomachic (13), antioxidant, anti-tumor (14) and mild laxative. It has been helpful in various conditions particularly asthma, piles and cough. It is also useful in healing of wounds and scalds. It can be used as gargle against inflammation of mucous

membrane in the mouth (15). *Terminalia cheubla* is also used for ulcers (16), vomiting, colic pain urinary infections, diabetes, skin diseases, parasitic infections, heart diseases, and hemorrhoids (15).

The aim of the present study was to investigate and to compare the mucosal protective effect of oral monotherapy with ethanolic extracts of chamomile and Haritaki with Asacol, as a reference anti-inflammatory drug in the experimental model of GAA5% induced UC in rabbits.

## **Materials and Methods:**

### **Animals:**

Twenty four healthy, local, domestic rabbits weighing (750-1600) gm of both sexes were used in this study. They were supplied by the local animal market at Al-Hilla city. All rabbits were housed two per cage, which was provided with a wire mesh floor. They were fed standard oxid pellets and given water *ad libitum*.

### **Medicinal plants:**

Flowers of *Matricaria chamomilla* (Chamomile) and fruits of *Terminalia chebula* (Haritaki) were dried and used in the present study. These plant materials were purchased from the local market and identified by the National Iraqi Institute for Herbs, Baghdad, Iraq.

### **Preparation of plant extract:**

Ethanolic extracts were accomplished according to the method of le Grand (17). Briefly 50 gm of each powdered plant sample

was mixed with 250 ml of 96% ethanol. The mixture was kept for 2-5 days in tightly sealed containers at room temperature and shaken several times daily. This mixture was filtered through filter paper to remove the coarse plant materials. Further extraction of the residue was repeated 3-5 times until a clear supernatant extraction liquid was obtained. The filtrates of each tested plant were evaporated to dryness using a rotary evaporator at 40°C. The final dried samples were weighed and stored at -20°C until use.

#### **Experimental design and animal grouping:**

Animals were divided into 4 groups (six animals in each group). Ulcerative colitis was induced in each group by using a method of Jabir (18). In brief, Five ml of glacial acetic acid (GAA) was diluted in a volumetric flask with 20 ml of distilled water, then 30 ml of 99.8% ethanol was added to the flask, and then distilled water (DW) was added to a final volume of 100 ml. this solution was kept in a glass container away from light and sealed tightly, to be used for induction of colitis. All the animals were food withheld 24 hours before induction of colitis. The animal was anaesthetized by using of chloroform soaked cotton piece applied over its nose and stretched straight on the table then a rubber catheter (2 mm outer diameter) was inserted into the lumen of the colon via the anus. The catheter was advanced so that the tip

was about 8 cm proximal to the anus (19). One ml of GAA 5% was instilled into the lumen of the colon using a syringe connected to the catheter. The animal was kept stretched (in a horizontal position) 10 to 20 seconds to prevent rapid spillage of the solution. Then the animal was transferred to its cage. One hour later, all the animals were allowed to have free access to food and water. The first group (control group) was received 10 ml of DW orally as a single daily dose. The second group was given Asacol (Mesalazine (Asacol)<sup>®</sup> : 800 mg enteric coating tablets-10 tablets, Tillotts Pharma-Switzerland) at a dose of 800 mg/kg BW dissolved in 10 ml of DW and administered as a single daily dose orally (Pretreatment was to enhance tissue repairing and to prevent or attenuate the severity of colonic ulceration that could be later produced by GAA 5%). The third group was given ethanolic extract of chamomile at a dose of 500 mg/kg BW dissolved in 10 ml of DW administered as a single daily dose orally. And finally the fourth group was given ethanolic extract of Haritaki at a dose of 1000 mg/kg BW dissolved in 10 ml of DW administered as a single daily dose orally.

Each agent (including DW) was administered two days prior to induction of colitis, the day of induction, and a dose 22 hours post-induction (i.e., 2 hours prior to sacrificing of the animal).

**Blood sampling:**

Blood samples were taken for biochemical and haematological analysis at 2 occasions:-

**1-**at the beginning of experiment (before induction of UC) to determine the normal values of serum sodium, serum potassium, total WBC, lymphocyte count, RBC count, HGB, and PCV% of the tested animals.

**2-**at the end of experiment (i.e., 24 after induction of UC)

Blood samples were obtained from the heart; 5 ml of blood could be aspirated in each occasion.

**Biochemical examination of sera:**

Serum sodium and potassium were estimated by using of flame photometer (ELICO<sup>®</sup>, CL378-England) according to the method of (20).

**Haematological assessment:**

Estimation of total WBC count has been carried out using hemocytometer with Neubaur improved double slide and Thomas's solution as a diluent (21). Where as lymphocytes count has been calculated in Leishman's stained blood smears by using of oil immersion (21). Estimation of RBC has been carried out using hemocytometer and Hayem's fluid as a diluent (20). HGB was tested by using of Sahli's hemoglobinometer method (Haemometer: MAREINFELD Laboratory glassware-Germany) (22). PCV% was assessed by using of haematocrit centrifuge (HAEMATOKRIT 20, Hettich ZENTRIFUGEN-Germany).

**Colon isolation and preparation:**

At the end of experiment, the animals were sacrificed, the abdomen was opened longitudinally, and a segment of colon 8 cm proximal to the anus was removed for assessment of colonic inflammation and ulceration. The excised colonic segment was immediately immersed in normal saline, cleaned from adherent tissues, opened longitudinally and then rinsed with 0.9% sodium chloride solution to discard the fecal materials (18).

**Gross examination:**

The segment was put on a clean white tray and examined grossly with the naked eye in order to examine the changes.

**Histopathological examination:**

After gross examination of the colon segments, they were placed in formalin 10% to be ready for sectioning which was carried out at Al-Karameh Hospital. The histopathological examination of the sections was then done to check the microscopic changes of the colon tissue by using light microscopy.

**Statistical analysis:**

The values are given as mean $\pm$ SE,  $P < 0.05$  and  $P < 0.01$  were used as a criterion for significance. The data were analyzed by student's *t*-test using SPSS (Version 10).

**Results:**

By the end of experiments, i.e., 24 hours after the rabbits being intrarectally administered by 1 ml of GAA 5%, colonic inflammation and

ulceration occurred in all of the studied animals with severity and extent that varied from group to another and the following results were detected:

#### **Serum electrolyte levels (sodium and potassium):**

Control group showed significant reduction in the levels of serum sodium and serum potassium at  $p < 0.05$  ( $150.6 \pm 12.63$  mmol/l,  $0.2 \pm 0.03$  mmol/l respectively) revealing hyponatraemia and hypokalaemia in comparison to the normal values ( $423.63 \pm 40.85$  mmol/l,  $0.98 \pm 0.07$  mmol/l respectively).

Asacol group showed significant elevation in the levels of serum sodium and serum potassium at  $p < 0.05$  ( $306.33 \pm 13.47$  mmol/l,  $0.58 \pm 0.03$  mmol/l respectively). Chamomile group showed significant elevation in the levels of serum sodium and serum potassium at  $p < 0.05$  ( $309.58 \pm 22.54$  mmol/l,  $0.51 \pm 0.04$  mmol/l respectively). Where as Haritaki group showed significant elevation in the levels of serum sodium and serum potassium at  $p < 0.05$  ( $318.91 \pm 25.60$  mmol/l,  $0.53 \pm 0.07$  mmol/l respectively) when compared with the control group (Table 1).

#### **Total WBC count and lymphocyte count:**

In control group, GAA 5% produced severe inflammation of colon assessed by significant elevation in the total WBC and lymphocyte count at  $p < 0.05$  ( $3.18 \pm 0.45$   $\times 10^3/\mu\text{l}$ ,  $1.68 \pm 0.23$

$\times 10^3/\mu\text{l}$  respectively) in comparison to the normal values ( $1.4 \pm 0.03$   $\times 10^3/\mu\text{l}$ ,  $0.73 \pm 0.03$   $\times 10^3/\mu\text{l}$ ). Asacol group showed significant reduction in the total WBC and lymphocyte count at  $p < 0.05$  ( $2.01 \pm 0.10$   $\times 10^3/\mu\text{l}$ ,  $0.96 \pm 0.05$   $\times 10^3/\mu\text{l}$  respectively). Chamomile group showed significant reduction in the total WBC and lymphocyte count at  $p < 0.01$  ( $2.5 \pm 0.07$   $\times 10^3/\mu\text{l}$ ,  $1.28 \pm 0.06$   $\times 10^3/\mu\text{l}$  respectively). While, Haritaki group showed significant reduction in the total WBC and lymphocyte count at  $p < 0.01$  ( $2.41 \pm 0.12$   $\times 10^3/\mu\text{l}$ ,  $1.25 \pm 0.07$   $\times 10^3/\mu\text{l}$  respectively) when compared with the control group (Table 2, 3).

#### **RBC count, HGB level, and PCV%:**

Control group revealed significant reduction in the count of RBC, HGB level, and percentage of PCV% at  $p < 0.05$  ( $4.35 \pm 0.09$   $\times 10^6/\mu\text{l}$ ,  $9.25 \pm 0.09$  gm/dl,  $32.5 \pm 0.67\%$  respectively) when compared with the normal values ( $6.08 \pm 0.08$   $\times 10^6/\mu\text{l}$ ,  $14.4 \pm 0.14$  gm/dl,  $60.16 \pm 2.17\%$  respectively). Asacol group showed significant elevation in the RBC count, HGB level, and percentage of PCV% at  $p < 0.05$  ( $5.11 \pm 0.07$   $\times 10^6/\mu\text{l}$ ,  $12.75 \pm 0.10$  gm/dl,  $45 \pm 0.96\%$  respectively). Chamomile group showed significant elevation in the RBC count, HGB level, and percentage of PCV% at  $p < 0.05$  ( $4.92 \pm 0.03$   $\times 10^6/\mu\text{l}$ ,  $12.58 \pm 0.15$  gm/dl,  $42 \pm 0.51\%$  respectively). And finally, Haritaki group showed significant

elevation in the RBC count, HGB level, and percentage of PCV% at  $p < 0.05$  ( $5.02 \pm 0.14 \times 10^6/\mu\text{l}$ ,  $12.7 \pm 0.07 \text{ gm/dl}$ ,  $48.83 \pm 1.14\%$  respectively) (Table 4, 5, 6).

#### **Gross examination:**

The induced histological damage was grossly obvious in the control group; it showed clear ulcer, severe erosion of the tissue, severe congestion, and severe hemorrhage (Figure 2, 3). Colon segments of Asacol group showed no ulcer, no erosion, mild congestion, and no hemorrhage (Figure 4). Where as colon segments of the Chamomile and Haritaki groups showed erosion, congestion, and mild hemorrhage without ulceration (Figure 5, 6).

#### **Histopathological examination:**

Histological sections of the control group showed severe complete sloughing of epithelium and sever hemorrhage in sub-epithelial layer (Figure 8,9). Colon of Asacol group showed regeneration of epithelium, mild fibrosis in sub-epithelial layer and hyperplasia in smooth muscles (Figure 10, 11). Colon of the Chamomile group showed less severe changes, when compared to the control group, but complete sloughing of epithelium and mild hemorrhage in sub-epithelial layer could be seen (Figure 12, 13). Colon of the Haritaki group showed complete regeneration and hyperplasia of epithelium congestion and severe fibrosis in sub-epithelial layer (Figure 14, 15).

**Table (1):** Serum sodium and potassium levels ( $M \pm SE$ ,  $n=6$ ) of control and treatment groups, measured pre- and post-induction of UC by GAA 5%.

Group	Serum Sodium level (mmol/l)		Serum Potassium level (mmol/l)	
	Pre-induction	Post-induction	Pre-induction	Post-induction
Control	$423.63 \pm 40.85$	$150.6 \pm 12.63$	$0.98 \pm 0.07$	$0.2 \pm 0.03$
Asacol	$423.63 \pm 40.85$	$306.33 \pm 13.47$ *	$0.98 \pm 0.07$	$0.58 \pm 0.03$ *
Chamomile extract	$423.63 \pm 40.85$	$309.58 \pm 22.54$ *	$0.98 \pm 0.07$	$0.51 \pm 0.04$ *
Haritaki extract	$423.63 \pm 40.85$	$318.91 \pm 25.60$ *	$0.98 \pm 0.07$	$0.53 \pm 0.07$ *

\*Significant elevating effect at  $p < 0.05$

**Table (2):** Total WBC count (M±SE, n=6) of the control and treatment groups measured pre- and post-induction of UC by GAA 5%.

Group	Total WBC count (X10 <sup>3</sup> /μl)	
	Pre-induction	Post-induction
Control	1.4±0.03	3.18±0.45
Asacol	1.4±0.03	2.01±0.10 *
Chamomile extract	1.4±0.03	2.5±0.07 **
Haritaki extract	1.4±0.03	2.41±0.12 **

\* Significant lowering effect at p<0.05.

\*\* Insignificant lowering effect at p<0.05, but it have significant lowering effect at p<0.01.

**Table (3):** Lymphocyte count (M±SE, n=6) of the control and treatment groups measured pre- and post-induction of UC by GAA 5%.

Group	Lymphocyte count (X10 <sup>3</sup> /μl)	
	Pre-induction	Post-induction
Control	0.73±0.03	1.68±0.23
Asacol	0.73±0.03	0.96±0.05 *
Chamomile extract	0.73±0.03	1.28±0.06 **
Haritaki extract	0.73±0.03	1.25±0.07 **

\* Significant lowering effect at p<0.05.

\*\* Insignificant lowering effect at p<0.05, but it have significant lowering effect at p<0.01.

**Table (4):** RBC count (M±SE, n=6) of the control and treatment groups measured pre- and post-induction of UC by GAA 5%.

Group	RBC count (X10 <sup>6</sup> /μl)	
	Pre-induction	Post-induction
Control	6.08±0.08	4.35±0.09
Asacol	6.08±0.08	5.11±0.07 *
Chamomile extract	6.08±0.08	4.92±0.03 *
Haritaki extract	6.08±0.08	5.02±0.14 *

\*Significant elevating effect at p<0.05.



**Table (5):** HGB level ( $M \pm SE$ ,  $n=6$ ) of the control and treatment groups measured pre- and post-induction of UC by GAA 5%.

Group	HGB level (gm/dl)	
	Pre-induction	Post-induction
Control	14.4±0.14	9.25±0.09
Asacol	14.4±0.14	12.75±0.10 *
Chamomile extract	14.4±0.14	12.58±0.15 *
Haritaki extract	14.4±0.14	12.7±0.07 *

\* Significant elevating effect at  $p < 0.05$ .

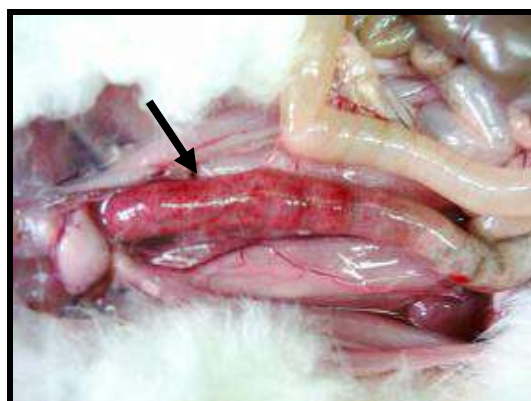
**Table (6):** Percentage of PCV ( $M \pm SE$ ,  $n=6$ ) of the control and treatment groups measured pre- and post-induction of UC by GAA 5%.

Group	PCV%	
	Pre-induction	Post-induction
Control	60.16±2.17	32.5±0.67
Asacol	60.16±2.17	45±0.96 *
Chamomile extract	60.16±2.17	42±0.51 *
Haritaki extract	60.16±2.17	48.83±1.14 *

\* Significant elevating effect at  $p < 0.05$ .



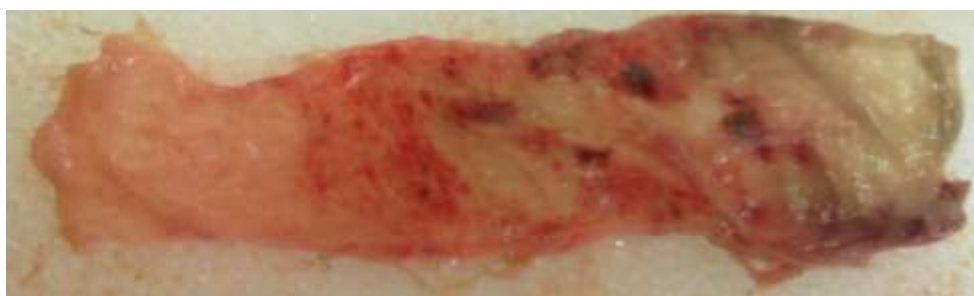
**Figure (1):** normal rabbit colon segment.



**Figure (2):** rabbit colon seen at laboratory of the control group showed severe inflammation, severe congestion, and hemorrhage.



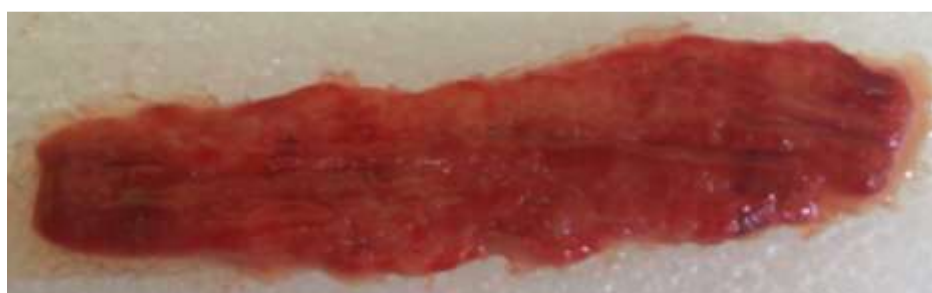
**Figure (3):** rabbit colon segment of the control group showed ulcer, severe erosion of the tissue, severe congestion, and severe hemorrhage caused by GAA5%.



**Figure (4):** rabbit colon segment of the group treated by Asacol showed no ulcer, no erosion, mild congestion, and no hemorrhage.



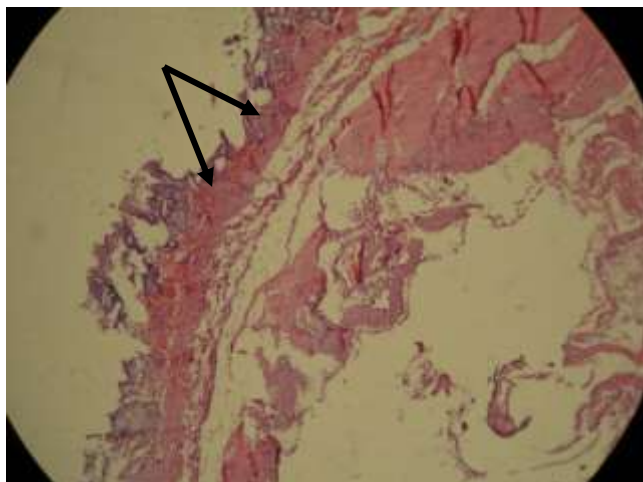
**Figure (5):** rabbit colon segment of the group treated by ethanolic extract of *Matricaria chamomilla* showed erosion, congestion, and mild hemorrhage without ulceration.



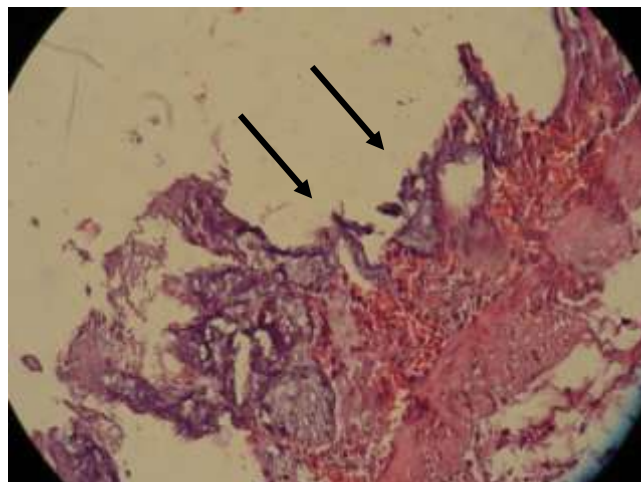
**Figure (6):** rabbit colon segment of the group treated by ethanolic extract of *Terminalia chebula* showed erosion, congestion, and mild hemorrhage without ulceration.



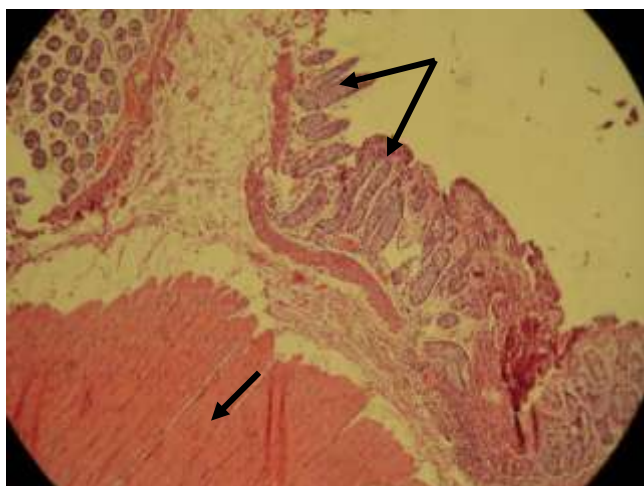
**Figure (7):** normal rabbit colon showed normal intestinal tissue, normal epithelium (arrow). 50X H&E stain.



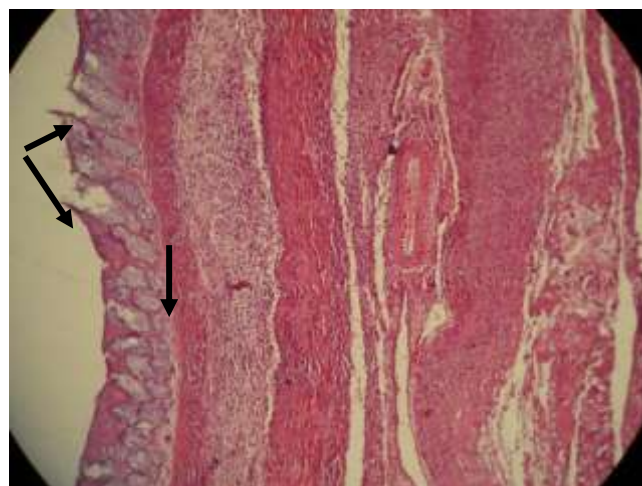
**Figure (8):** colon of the control group showed severe complete sloughing of epithelium (arrows) and hemorrhage in sub-epithelial layer. 50X H&E stain.



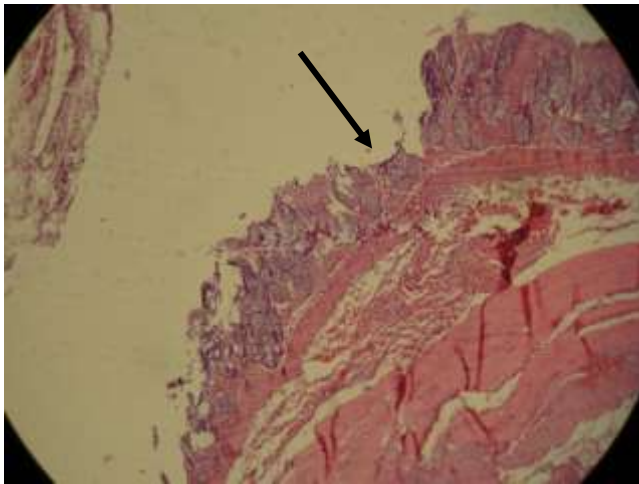
**Figure (9):** colon of the control group showed severe complete sloughing of epithelium (arrows) and severe hemorrhage in sub-epithelial layer. 50X H&E stain.



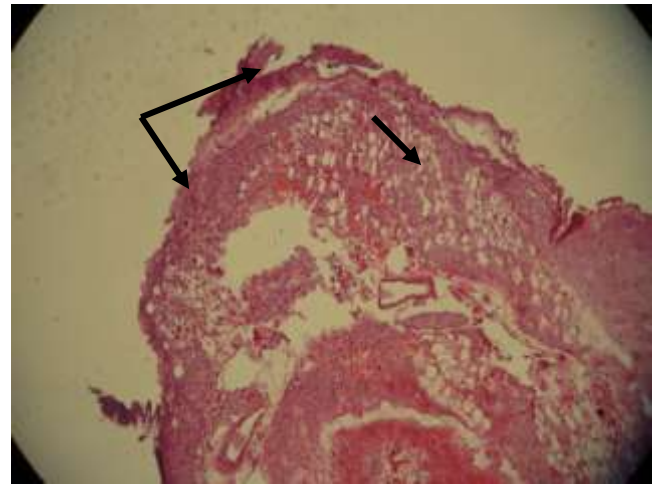
**Figure (10):** colon of the Asacol group showed regeneration of epithelium (arrows), congestion in sub-epithelial layer and hyperplasia in smooth muscles. 50X H&E stain.



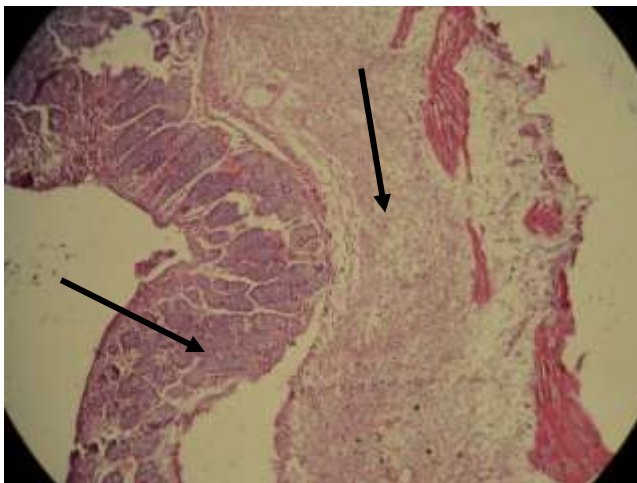
**Figure (11):** colon of Asacol group showed regeneration of epithelium (arrows), mild fibrosis in sub-epithelial layer (arrow) and hyperplasia in smooth muscles. 50X H&E stain.



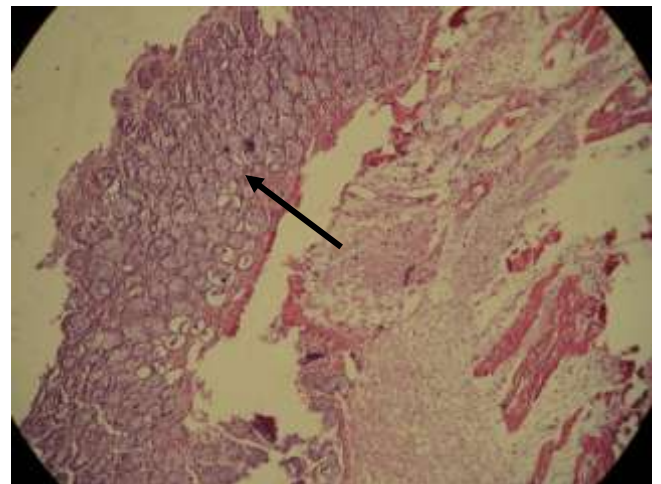
**Figure (12):** colon of the Chamomile group showed less severe, but complete sloughing of epithelium (arrow) and mild hemorrhage in sub-epithelial layer (arrow) . 200X H&E stain.



**Figure (13):** colon of the Chamomile group showed less severe, but complete sloughing of epithelium (arrows), hemorrhage and congestion in sub-epithelial layer (short arrow). 200X H&E stain.



**Figure (14):** colon of Haritaki group showed complete regeneration and hyperplasia of epithelium (arrows), congestion and severe fibrosis in sub-epithelial layer.50X H&E stain



**Figure (15):** colon of the Haritaki group showed complete regeneration and hyperplasia of epithelium (arrow), congestion and severe fibrosis in sub-epithelial layer. 50X H&E stain.

## Discussion:

Various animal models have provided a foundation for future investigation into the mechanisms responsible for UC, which will hopefully result in the development and testing of novel therapeutic regimens (23). Glacial acetic acid-induced colitis is used widely because of its reproducibility (with lesions occurring in 100% of animals). In addition, it provides an inexpensive model useful in comparing the effectiveness of novel therapeutic agents (24). Its similarity with human UC in many aspects make researchers still use it as one of the models of induced colitis. In the present study, ethanol was used in combination with acetic acid (5%GAA-30% Ethanol) in order to decrease the mucosal barrier (18).The schedule of therapy (2 days before, the day of induction, and 22 hours after induction of colitis by GAA5%) was dependent in this study to evaluate mainly the possible prophylactic role of the tested agents in addition to their effectiveness in initial therapy for acute attacks of colitis. Blood samples were collected from all of the tested rabbits prior to the beginning of experiment and in the last day, the day of animal sacrificing. Measurement of serum electrolytes (sodium and potassium), haematological parameters (total WBC count, lymphocyte count, RBC count, HGB, PCV%) besides gross and microscopical examination of colon sections was dependent to

assess the severity of symptoms of UC among the tested groups (control and treatment groups). The present study showed that the rectal administration of 1 ml of 5%GAA-30%ethanol to the control group could produced hyponatraemia, hypokalaemia, severe inflammation indicated by leukocytosis and lymphocytosis, also it induced congestion and hemorrhage revealed by erythrocytopenia, low HGB level and low PCV%. Where as gross examination of colon segment showed severe ulceration, erosion, and hemorrhage. These symptoms were similar to those mentioned in the diagnosis of UC in the review of literatures in addition to the histopathological changes that were proven by Jabir (18).

Mesalazine or called Asacol (active metabolite of sulfasalazina), the oral anti-inflammatory-delayed release tablets used commonly as a standard therapy to control acute attacks of UC. It is also thought to be an antioxidant that traps free radicals, which are potentially damaging byproducts of metabolism so it was used in the present study as what is called a positive control. Because the dose of oral Asacol in human is 1.6 gm divided into two times (25, 26) and the reproduction studies with sulfasalazina compounds in rats and rabbits at doses of 800 mg/kg BW or up to six times the human dose have not shown impaired female fertility or harmful effects (27, 28), so this

pushed us to test several doses of Asacol, they were 200, 400, 800 mg/kg BW, but the most effective dose in reducing damaging effect of GAA5% on rabbit colon was 800 mg/kg BW/day p.o, where as the others were discluded. Oral administration of Asacol had significantly elevated serum electrolyte levels, decreased inflammation, reduced blood loss, and prevented the colonic ulceration. Also the present study indicates that slow release 5-amino-salicylic acid at the larger dose reaches the large bowel in sufficiently high concentrations following oral administration and significantly reduces GAA5%-induced colitis in the rabbits when compared with the control group and other treatment groups. Kitano et al. ,(29) had demonstrated that Mesalazine microgranules (50 or 150 mg/kg BW/day) which were administered orally to each rabbit with carrageenan-induced colitis for six weeks, showed inhibitory effect on colonic mucosal damage.

Third group of rabbits were treated orally with ethanolic extract of Chamomile in various doses ranging from 300 mg/kg BW to 500 mg/kg BW. The effective dose was 500 mg/kg BW, since this dose elicited a maximum reduction in lesion severity and revealed accepted anti-ulcer effect, it produced significant elevation in the serum electrolyte levels, reduce inflammation and degree of hemorrhage as it assessed by

biochemical and haematological tests, as well as it reduced the gross and microscopical changes characteristic of experimental UC. Anti-ulcer effect of chamomile was proven by Karbalay-Doust and Noorafshan (11), through using its aqueous extract at a dose of 400 mg/kg BW p.o in an experimental model of gastric ulcer in mice. Chamomile extract contains many components that may exert anti-ulcer effects. Amino acids, polysaccharides, and fatty acids are some of its constituents. The flowers of chamomile contain 1-2% volatile oils including  $\alpha$ -bisabolol,  $\alpha$ -bisabolol oxides A & B, matricine, a variety of mineral elements including manganese and magnesium (30). Flavonoids and other phenolic compounds have been identified in various parts of the chamomile flower head. Apigenin, quercetin, patuletin, luteolin and their glucosides are the major flavonoids present in the flower. The presence of large amounts of cinnamic acid derivatives, frolic and caffeic acid, as well as other unidentified phenolic derivatives of the total flower has been investigated. All of the constituents, which have also been found in other plants, may have therapeutic effects (11). Here we reported the anti-ulcer effects of chamomile flower extract on acute experimental UC in rabbits. Our results are consistent with Khayyal and co-workers (31), they have shown that the extracts from the plant *Matricaria recutita* alone

or in combination with other plants have antiulcer activity. They have reported that the cytoprotective effect of the herbal extracts could be partly caused by their flavonoid content and to their free radical scavenging properties.

Ethanol extract of Haritaki was given to the animals (fourth group) at a different doses 500 mg/kg BW, and 1000 mg/kg BW daily via oral route, but the effective dose was 1000 mg/kg BW because it produced significant increment in the levels of serum sodium and potassium, besides significant reduction in the inflammation by its anti-inflammatory activity, and desirable mucosal protective effect against the ulcerogenic action of GAA5% on the colon of experimental animals. The mucosal protective and anti-ulcer properties of Haritaki extract were demonstrated by Devi et al., (16) who found that the treatment of rats with methanolic extract of Haritaki at a dose of 500 mg/kg BW/day p.o could produced significant reduction in lesion index in rats suffering from diclofenac-induced gastric ulcer confirming the gastroprotective activity of Haritaki extract which probably related to its free radical scavenging activity, antioxidant affectivity, and cytoprotective nature. So Hazra et al., (15) had proven the scavenging activities of the methanolic extract of Haritaki fruits which contains significant amount of phenolic contents including ellagic acid, 2,4-chebulyl-D-glucopyranose,

chebulinic acid, gallic acid, ethyl gallate, punicalagin, terflavin A, terchebin, luteolin, and tannic acid.

These compounds have good antioxidant potential and their effects on human nutrition and health are considerable. Phenolic contents are also very important plant constituents because of their scavenging ability due to their hydroxyl groups (15).

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