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Potential Anti inflammatory Effect of Statin on Inflammatory Colitis Induced in Experimental Animals

A Thesis Submitted to College of Medicine and Committee of Postgraduate Studies of University of Babylon as Partial Fulfillment of the Requirements for the Degree of Master in Pharmacology and Toxicology

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بسم الله الرحمن الرحيم

((إِلَّا أَنْ يَشَاءَ اللَّهُ ۚ نَرْفَعُ دَرَجَاتٍ مَنْ نَشَاءُ ۗ وَفَوْقَ كُلِّ ذِي عِلْمٍ عَلِيمٌ))

صدق الله العظيم

سورة يوسف الآية(76)

Dedication

- *To My Great Father And Mothert*
- *To My Faithful Husband, And My Beloved Sons Whose Sacrifices, Which Were Realized By Our Loss Of Precious Time Together*
- *To My Brothers and Sisters*
- *Lastly To All My Teachers ….*

Zeena hadi al yassery

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Examining Committee Certificate

We certify that we have read this thesis entitled "**Potential Anti inflammatory Effect of Statin on Inflammatory Colitis Induced in Experimental Animal**" and as an examining committee examined the student "**Zeena Hadi Mageed**" in its contents and that in our opinion it meets the standard of a thesis for the degree of Master in Pharmacology and Toxicology.

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Summery

Ulcerative colitis (UC), first described in 1859, is one of two major forms of inflammatory bowel disease (IBD). UC is characterized by mucosal inflammation initiating in the rectum and extending proximally in the colon in a continuous fashion. The exact pathogenesis of UC is still unknown but several factors, including a dysregulated immune response, altered gut microbiota, genetic susceptibility and environmental factors, have been implicated . A bloody diarrhoea is the most common symptom of UC, although diagnosis is made from a combination of symptoms, endoscopy and histology.

The increased proportion of undesirable effects at the same time with insufficient therapeutic effectiveness of current treatment, indicate that searching for new drugs is necessary probably with less adverse effects and high effectiveness . This study is to investigate the effect of atorvastatin and rosuvstatin on inflammatory biomarkers , oxidative stress parameters and histological outcome in experimentally induced colitis .

This study was conducted on 84 adult male mice weighing 25-30g previously submitted to starvation for at least 24hrs .Animals were divided into seven groups (n=14/group except control group=10) .All groups except group I (act as normal control and received no treatment) were received 5ml /kg acetic acid rectally .Group II served as a colitis were received only acetic acid rectally. Group III with colitis treated with 5mg atorvastatin , IV with colitis treated with 50mg atorvastatin, V with colitis treated with 2mg rosuvastatin and VI with colitis treated with 10 mg rosuvastatin respectively for 7days.On the other hand Group VII served as positive control receiving 1mg/kg prednisolone orally as a standard therapy for 7days. All these drugs were given for seven days orally by using oral

gavage. At the end of the experimental period, animals were sacrificed by an over dose of chloroform inhalation. The colon was removed rapidly after dissection of the abdomen. The specimen of colon was opened longitudinally and gently cleaned with normal saline. Then, the assessment of clinical parameters, histopathologic changes and biochemical expression of colonic cytokines (C-reactive protein ,tumor necrosis factor-alpha and interleukin-4), and oxidative stress markers (myeloperoxidase).

Results showed that rectal administration of 5ml /Kg of 4% acetic acid in rats caused 100% induction with significant increase in clinical, histological bio chemical ,and oxidative stress parameters compared to control normal group. Statin (atorvastatin and rosuvastatin)significantly reduced the clinical, histo pathological score , biochemical and oxidative stress parameters in comparison with acetic acid induced colitis group. Statin (atorvastatin and rosuvastatin) in high dose produced the most significant reduction in clinical, macroscopic score, histo pathological score, tumor necrosis factor-alpha, interleukin-6,C-reactive protein and myeloperoxidase means compared to low dose of these drugs. Atorvastatin had statistically higher effect in reducing the disease activity index, colon weight means, histo ,chemical(tumor necrosis factor-alpha, interleukin-6),and myeloperoxidase compared with rosuvastatin.

In conclusion, our study had shown that atorvastatin, and rosuvastatin have a therapeutic role on the experimentally acetic acid induced colitis in mice. The most useful drug is atorvastatin in high dose and its effect was comparable to that produced by prednisolone through anti-inflammatory and antioxidant actions in ulcerative colitis.

الخلاصه

تم وصف التهاب القولون التقرحي (UC) لأول مرة في عام 1859 ، وهو أحد شكلين رئيسيين لمرض التهاب الأمعاء .(IBD) يتميز التهاب القولون التقرحي بالتهاب الغشاء المخاطي الذي يبدأ في المستقيم ويمتد بالقرب من القولون بشكل مستمر. لا يزال التسبب الدقيق لمرض UC مجهولًا ، ولكن هناك عدة عوامل متورطة ، بما في ذلك الاستجابة المناعية غير المنتظمة ، وتغيير الميكروبات المعوية ، والقابلية الوراثية ، والعوامل البيئية. الإسهال الدموي هو أكثر أعراض شيوعًا لالتهاب القولون التقرحي ، على الرغم من أن التشخيص يتم من خلال مجموعة من الأعراض والتنظير الداخلي وعلم الأنسجة.

تشير النسبة المتزايدة من الآثار غير المرغوب فيها في نفس الوقت مع الفعالية العلاجية غير الكافية للعلاج الحالي إلى أن البحث عن أدوية جديدة ضروري على الأرجح مع تأثيرات ضائرة أقل وفعالية عالية. تهدف هذه الدراسة إلى التحقق من تأثير أتورفاستاتين وروزوفستاتين على المؤشرات الحيوية الالتهابية ومعايير الإجهاد التأكسدي والنتائج النسيجية في التهاب القولون المستحث تجريبياً.

أجريت هذه الدراسة على 84 فأراً بالغاً يتراوح وزنها بين 25 و 30 جراماً تعرضت سابقاً للمجاعة لمدة 24 ساعة على الأقل ، وقسمت الحيوانات إلى سبع مجموعات (ع = 14 / مجموعة عدا المجموعه الموجبه=10).) تم تناول 5 مل / كجم من حمض الأسيتيك عن طريق المستقيم ، أما المجموعة الثانية التي استخدمت على شكل التهاب القولون فقد تم تناول حمض الأسيتيك عن طريق المستقيم فقط. المجموعة الثالثة مع التهاب القولون تعامل مع 5 ملغ أتورفاستاتين ، IV مع التهاب القولون تعامل مع 50 ملغ أتورفاستاتين ، V مع التهاب القولون تعامل مع 2 ملغ روسوفاستاتين و VI مع التهاب القولون المعالج بـ 10 ملغ روسوفاستاتين على التوالي لمدة 7 أيام. بريدنيزولون شفويا كعلاج قياسي لمدة 7 أيام. تم إعطاء كل هذه الأدوية لمدة سبعة أيام عن طريق الفم

في نهاية الفترة التجريبية ، تم التضحية بالحيوانات بجرعة زائدة من استنشاق الكلوروفورم. تمت إزالة القولون بسرعة بعد تشريح البطن. تم فتح عينة القولون طوليا وتنظيفها برفق بمحلول ملحي عادي. ثم تقييم المؤشرات السريرية والتغيرات النسيجية والتعبير البيوكيميائي للسيتوكينات القولونية (بروتين سي التفاعلي ، عامل نخر الورم ألفا وإنترلوكين -6) ، وعلامات الإجهاد التأكسدي (الميلوبيروكسيديز).

أظهرت النتائج أن إعطاء 5 مل / كغ من حمض الخليك 4٪ في المستقيم في الجرذان تسبب في تحريض 100٪ مع زيادة مشخصه في معاملات الإجهاد الإكلينيكي والنسيجي والتأكسدي مقارنة بالمجموعة العادية الضابطة. الستاتين (أتورفاستاتين وروسيوفاستاتين)يخفض بشكل ملحوظ من الدرجة السريرية ، والنتيجة المرضية للنسيج ، ومعايير الإجهاد الكيميائي الحيوي والأكسدة بالمقارنة مع مجموعة التهاب القولون الناجم عن حمض الأسيتيك. أنتج الستاتين (أتورفاستاتين وروسيوفاستاتين) بجرعة عالية أكبر انخفاض في النتيجة السريرية والعيانية والنتيجة المرضية للنسيج وعامل نخر الورم ألفا وإنترلوكين 6 والبروتين التفاعلي C و myeloperoxidase مقارنة بالجرعة المنخفضة من هذه الأدوية. كان لأتورفاستاتين اعلى تأثير إحصائياً في خفض مؤشر نشاط المرض ووزن القولون وكذتك ، هيستو ،معايير كيميائية (عامل نخر الورم ألفا ، إنترلوكين -6) ، وميلوبيروكسيديز مقارنة مع روسوفاستاتين.

في الختام ، أظهرت دراستنا أن للأتورفاستاتين والروسوفاستاتين دور علاجي في التهاب القولون الناجم عن حمض الأسيتيك تجريبياً في الفئران. أكثر الأدوية فائدة هو أتورفاستاتين بجرعة عالية وكان تأثيره مشابهًا لما ينتج عن بريدنيزولون من خلال الإجراءات المضادة للالتهابات ومضادات الأكسدة في التهاب القولون التقرحي.

This study was conducted on 70 adult male mice weighing 25-30g previously submitted to starvation for at least 24hrs .Animals were divided into seven group (n=10/group) .All groups except group I (act as normal control and received no treatment) were received 5ml /kg acetic acid rectally .Group II served as a colitis were received only acetic acid rectally. Group III with colitis treated with 5mg atorvastatin , IV with colitis treated with 50mg atorvastatin, V with colitis treated with3mg rosuvastatin and VI with colitis treated with 10 mg rosuvastatin respectively for 7days.On the other hand Group VII served as positive control receiving 10mg/kg prednislone orally as a standard therapy for 7days. All these drugs were given for seven days orally by using oral gavage. At the end of the experimental period, animals were sacrificed by an over dose of chloroform inhalation. The colon was removed rapidly after dissection of the abdomen. The specimen of colon was opened longitudinally and gently cleaned with normal saline. Then, the assessment of clinical parameters, histopathologic changes and biochemical expression of colonic cytokines (C-reactive protein ,tumor necrosis factor-alpha and interleukin-4), and oxidative stress markers (myeloperoxidase).

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List of Abbreviations

Abbrevaition	Description
A-A	Acetic acid
ACG	American Collage Gastro neterology
ADL	Adalimumab
APC	Anti gen –Presenting Cell
Ator	Atorvastatin
CD62	Cluster of differentiation 62
CMV	Cytomegalovirus

Cont	Control
CRP	C-Reactive Protein
CT	Computed Tomography
DIA	Disease Activity Index
E	Endothelial
E	Eosin
EIM	Extra Intestinal Manifestation
FC	Fecal Calprotectin
g	Gram
GIT	Gastro Intestinal Tract
GLM	Golimumab
H	Hematoxylin
HD	High Dose
HISTO	Histopath
HMG COA	Hydroxy-Methyl-Glutaryl-Coenzyme A
IBD	Inflammatory Bowel Disease
ICAM-1	Intracellular adhesion molecule
IG	Immunoglobulin
IL-6	Interleukin-6
Ind	Induction
INF-γ	Interferon -γ
Kg	Kilo gram
L	Leukocyte

LD	Low Dose
MABS	Monoclonal antibodies
MAC	Macroscopic
Mg	Milligram
MIC	Microscopic
ml	Milliliter
MPO	Myeloperoxidase
MRI	Magnetic Resonance Imaging
P	Platelet
Pred	Prednisolone
RNS	Reactive Nitrogen Species
Ros	Rosuvastatin
ROS	Reactive Oxygen Species
SD	Stander Deviation
SMC	Smooth Muscle Cell
T-h	T-helper
TNF-α	Tumor Necrosis Factor-α
UC	Ulcerative Colitis
VCAM-1	Vascular cell adhesion molecule
W/L	Weight/Length

1.1. Introduction

Inflammatory bowel disease (IBD) comprises those conditions characterized by a tendency for chronic relapsing remitting inflammatory disease of the large intestine (Safarpour *et al.*, 2013).

Inflammatory bowel disease is a progressive inflammatory intestinal disease characterized by colon tissue ulceration and increased epithelial permeability of the colon and extensive infiltration of leukocytes in the colon (Xiao *et al.*, 2013).

Crohn's disease (CD) and ulcerative colitis (UC) are the two major forms of IBD , which are both histologically and clinically non-identical (Ko, Lam and Cheung, 2005).

Nevertheless, these forms of IBD share many clinical and epidemiological characteristics, suggesting that the underlying cause may be similar (FRCS, 2008).

Ulcerative colitis mainly involves inflammation of the colon while in Crohn's disease the inflammation can involve any part of the gastrointestinal tract (GIT) from the mouth to the anus (Qureshi *et al.*, 2010) .However the distal small bowel and the colon are most commonly involved in Crohn's disease (Mills and Stamos, 2007).

About 10% to 20% of patients suffering from UC have a major problem that this disease itself leads to colon or bowel cancer with 10 – 20 times (Thippeswamy *et al.*, 2011); (Patil, Kandhare and Bhise, 2012).

Inflammatory changes associated with UC are limited to the mucosa and typically affects the rectum but often extends to involve the whole colon in a continuous manner (Aleisa *et al.*, 2014).

1.2.Aim of Study

1) Investigate the effect of rosuvastatin and atorvastatin, on disease activity index, oxidative stress parameters , histological outcome and other inflammetory markers in experimentally induced colitis.
2) Found out the correlation between severity index with histopathology score, oxidative stress parameters and other inflammatory markers in animals induced colitis.
3) Compare the results obtained with rosuvastatin and atorvastatin with that of in animals induced colitis.

1.3.Literature Review

1.3.1.Ulcerative Colitis

Ulcerative colitis (UC) is a chronic inflammatory bowel disease (IBD) present with inflammation and ulceration of the rectum, extending as far as the cecum ’if the Disease involving only the most distal part of the colon and the rectum is termed ulcerative pro colitis; disease from the descending colon down is referred to as limited or distal colitis; and disease involving the entire colon is called pancolitis (Head and Jurenka, 2019).

The goal of care is to induce and maintain long-term remission as UC patients often experience a relapsing and remitting disease course (Head and Jurenka, 2019); (Stange *et al.*, 2008). This clinical course occurs either spontaneously or in response to treatment (Kornbluth and Sachar, 2010). Therefore, the evaluation of new treatments for UC include assessments of symptoms and more objective measures of the underlying disease process, such as endoscopy. For both these disease activity (total Mayo score) and endoscopic and healing are essential components of clinical trials in UC (Head and Jurenka, 2019);(Christensen *et al.*, 2017).

The significance of endoscopic mucosal healing is supported by its association with improved long-term outcomes in UC, but microscopic inflammation is common in endoscopically healthy mucosa, (Head and Jurenka, 2019) suggesting the cellular level assessment can provide an additional measurement of disease activity and response to treatment (Marchal-Bressenot *et al.*, 2017).

However, the definition of histologic improvement analogous to endoscopic improvement or clinical remission has not been exactly established. Ulcerative colitis and Crohn’s disease are chronic diseases, relapsing, immunologically mediated disorders that are convergent referred to as inflammatory bowel diseases (IBD) .The prevalence of IBD quickly increased in Europe and North America in the second half

of the twentieth century and is becoming more common in the rest of the world as various countries adopt a Western lifestyle. Such epidemiologic observations indicated that there are strong environmental influences on IBD: their influence is proved by the relatively low concordance rate in identical twins (~50% for Crohn's disease, and ~10% for ulcerative colitis) (Sartor, 2006). This study and propagate the incidence of IBD in first-degree relatives of probands with either disease indicated that genetic factors are also integrally involved. Even with this knowledge, the etiologies of these diseases remain a puzzle. In the past years, however, work in animal models, human genetics, basic science and clinical trials, have provided new acumen into the pathogenesis of these diseases. The most widely held hypothesis on the pathogenesis of IBD is that more aggressive acquired (T-helper cell) immune responses to a subset of commensal enteric bacteria developed in genetically susceptible hosts, and environmental factors precipitated the onset or reactivation of disease (Sartor, 2006).

1.3.1.1. Epidemiology

The occurrence of UC worldwide has increased over the last few years. In contrast to the developed countries of North America and Western Europe, where the incidence of UC has plateaued or even reduced, publications show that the number of cases has elevated in developing countries, as in the example in Latin America, Asia and Eastern Europe (Da Silva *et al.*, 2014). Despite the increased incidence in these places, there are still differences in UC incidence and prevalence in different regions of the world. The incidence rate of UC ranges from 0.5 to 31.5 per 100000 people each year, relying on the studied population (Burisch and Munkholm, 2013). The prevalence was lower in developing countries. For example in the Asia population, the prevalence varies from 5.3 to 63.6 per 100000 people (Niriella *et al.*, 2010), whereas in North American countries, it varies from 37.5 to 238 per 100000 people (Cosnes *et al.*, 2011).

Also the gradient between the occurrence of UC in the West and in Asian countries, it is noted that in Europe, although there are some exceptions, also there is a geographical gradient for the occurrence of IBD, with higher rates in the north and a lower frequency in the south (Da Silva *et al.*, 2014).

1.3.1.2.Etiology of ulcerative Colitis

Genetic predisposition, environmental factors and a dysbiotic microbiota with an excessive host response.

1.3.1.3. Symptoms of ulcerative Colitis

In begging the clinical presentation of patients with UC involves intestinal symptoms such as abdominal pain, diarrhoea and rectal bleeding, Extraintestinal manifestations in UC occur in nearly 30% of patients. They occur more commonly in patients with specific serologic markers, including HLA-DR. Extraintestinal manifestations (EIM) are repeatedly encountered. The most common EIM is located in the skin, eyes, liver, mouth and Joint .In (Joint) Arthritis may be axial or peripheral and involve either small or large joints. The existence and extent of arthritis and arthralgia often correlate with the activity of the bowel disease. Ankylosing spondylitis is uncommon during childhood but occurs in up to 6% of adults with UC. The oral cavity is also considered an extra-intestinal site of involvement in UC (Kaenkumchorn and Wahbeh, 2020).

At now studies have reported inconclusive results on the prevalence of oral and dental complaints in patients with UC. it is associated with specific and nonspecific oral signs and symptoms such as halitosis, dry mouth, aphthous ulcers, pyostomatitis vegetans and lichen planus. Some oral appearance seems specifically correlated with disease activity (Goldinova *et al.*, 2020).

Saliva is the main factor for the maintenance of oral and general homeostasis. It has a conclusive function in digestion, hydration of the oral mucosa and protection of

the teeth. Caries, periodontal disease and some oral inflammation may be caused by a lack of antimicrobial peptides present in saliva, sometimes occurring because of hyposalivation (Jager *et al.*, 2018). EIMs can affect other organ systems, in (Liver)such as the hepatobiliary (primary sclerosing cholangitis, autoimmune hepatitis), ocular (eye) such as (uveitis, episcleritis), cutaneous (skin) such as (erythema nodosum, pyoderma gangrenosum, and psoriasis), and hematologic (thrombosis) systems (Vavricka *et al.*, 2011). also, Primary sclerosing cholangitis is more common in males, patients with the more comprehensive disease, and pediatric patients (3.5% vs 9.8% in adults).Children with even moderate elevate in gamma-glutamyl transferase level at diagnosis are more likely to develop primary sclerosing cholangitis. Primary sclerosing cholangitis is the main risk factor for the development of cholangiocarcinoma and is associated with the higher happening of colorectal cancer (Lindberg *et al.*, 2008).

Also, the patients may have rectal bleeding, urgency, and tenesmus. In patients with proctocolitis or left-sided colitis, above 10% of cases can present with paradoxical constipation. In children, acute weight loss is common but the growth level is typically preserved, in contrast to Crohn's disease where linear growth is commonly affected (Kaenkumchorn and Wahbeh, 2020). Up to 15% of patients can at first present with severe UC, which may also have systemic symptoms such as fever, weight loss, tachycardia, nausea, and emesis (Magro *et al.*, 2017). The major concerning acute UC complication is toxic megacolon. Patients may initially be presented with toxic megacolon, although more commonly they increase toxic megacolon during a UC relapse episode (Gan, 2003) .

Clinically, the lineament of toxic megacolon aside from severe UC symptoms is colonic dilation on abdominal imaging. Toxic megacolon is a surgical emergency giving the risk of potential perforation and sepsis.

1.3.1.4. Pathogenesis

The exact cause of UC is still unknown but is thought to be due to a combination of a patient's genetics, microbiome interactions, immune response (disturbances in the innate and adaptive immune responses), and environmental factors (Ashry *et al.*, 2016).

A.Genetic Predisposition

The role of genetic factors has been strongly supported by epidemiologic and clinical studies (Ye and McGovern, 2016). Around 10-20% of patients will record a family history of IBD in a first-degree relative (Jostins *et al.*, 2012).

Genome-wide association studies (GWAS) have known over 200 genetic susceptibility loci associated with susceptibility to IBD with most genes contributing to both ulcerative colitis and Crohn's disease (Huang *et al.*, 2017). Many of these loci are detected in immune genes, including those concerned with innate and adaptive immune responses, and have been linked with other autoimmune diseases. While there are specific gene variants concerned with either CD or UC, the majority are shared by both forms of IBD (Jostins *et al.*, 2012);(Wang *et al.*, 2019). With the common use of genome-wide association studies (GWAS) and single nucleotide polymorphism (SNPs), a significant association between IBD and the interleukin 23 receptor (IL-23 R) gene encode a subunit of the receptor for IL- 23, the pro-inflammatory cytokines involved in the generation of T- helper17 cell by T-cells, and producing IL-17 which is well known to be involved in the inflammatory mechanism of IBD (Sandborn *et al.*, 2017);(Zhang and Li, 2014). The T-helper17 and IL23 pathway is well established in the pathogenesis of IBD, with susceptibility gene Loci IL23R, IL12B, Janus kinase 2 (JAK2) and signal transducers and activators of transcription-3 (STAT3) have been recognized in IBD (Zhang and Li, 2014).

B.Environmental factor

Many environmental exposures have been associated with UC. Smoking has been the most consistent and most studied environmental factor associated with UC. Interestingly, smokers have a decreased risk of developing UC (Disease and Guide, 2015), subsequent studies have confirmed the protective effect of heavy smoking on UC development with a lower rate of relapse (Ananthakrishnan, 2015).

Medications such as non-steroidal anti-inflammatory drugs (NSAIDs) have been associated with exacerbation of UC (Ananthakrishnan *et al.*, 2012). It is thought this may result from direct inhibition of the synthesis of cytoprotective prostaglandins, and use of these drugs is usually not recommended in patients with active disease (Zhang and Li, 2014).In addition, it was observed that antibiotic use was an important factor of UC onset as antibiotics could change the composition and amount of the intestinal microflora, which lead to irregular inflammatory responses (Hviid, Svanström and Frisch, 2011); (Ke *et al.*, 2017).

Demonstrated that vitamin D plays a major role in intestinal immunity and preservation of the epithelial barrier integrity, vitamin D insufficiency worsens UC and its supplement improves the disease in an animal study (Law *et al.*, 2019). Moreover, the increased vitamin D intake in animal models prevents inflammation-associated colon cancer, stress plays a role in the pathogenesis of UC (Mawdsley and Rampton, 2007).

Moreover, it was established that persons with a minimum level of stress had a lower risk of the disease onset (Bitton *et al.*, 2008). Evidence from animal models conducted that chronic psychological stress aggravates UC by upgrading damage to the colon mucosa, thereby disrupting barrier function (M'Koma, 2018).

The role of air pollutants as a risk factor of UC has been supported by epidemiologic evidence (Thia *et al.*, 2008). The particulate matter, nitrous oxides, or ozone in the air intrude into the intestine by food and water, which may cause increased epithelial

permeability of the gut, or stimulate an inflammatory response, thus leading to the occurrence of UC (Salim, Kaplan and Madsen, 2013); (Ke *et al.*, 2017).

Lastly, there is significant interest in the role of diet in the pathogenesis of IBD (Shivashankar and Lewis, 2017). A diet with an elevated level of protein or saturated fat has been variably correlated with ulcerative colitis, while a diet high in N-3 Polyunsaturated fatty acid manifests to minimize the risk of ulcerative colitis (Wędrychowicz, Zając and Tomasik, 2016).

C. Microbial factor

Many researchers have found gut microbiota to be closely associated with the onset and development of UC (Pei *et al.*, 2019). Under normal physiological conditions, the human gut microbiota is necessary for intestinal homeostasis with various vital functions and mutual relations important to host health including food digestion, development of the host immune system and intestinal epithelial barrier and defence against pathogens (Shreiner, Kao and Young, 2015); (Zuo *et al.*, 2018). Disruption of this equilibrium can result in dysbiosis and promote several gastrointestinal diseases like UC (Forbes, Van Domselaar and Bernstein, 2016); (Khan *et al.*, 2019).

Overexposure of the immune system in the presence of excessive bacterial substances could also show a loss of immunological tolerance to the bacteria, which are commonly considered as intestinal normal flora; furthermore, it may subsequently evolve bowel inflammation and UC development (Azimi *et al.*, 2018). Obviously, the predominant pathogens that could be associated with the UC disease Clostridium difficile (Hanada *et al.*, 2018), Escherichia coli, Listeria monocytogenes, Campylobacter and Bacteroides fragilis have been implicated in UC onset (Becker, Neurath and Wirtz, 2015).

D. Immunological Factors

The immune response has long been implicated in the pathogenesis of ulcerative colitis (Wang *et al.*, 2019). Evidence are suggested that the dysregulation of innate and adaptive immunity pathways participate in the intestinal inflammatory response in ulcerative colitis patients (Silva *et al.*, 2016).

Primarily, dysregulation of innate immunity in association with epithelial barrier dysfunction may be the basis for initiation of the mucosal inflammation, then the adaptive immunity which removes specific pathogens through humoral and cellular response has been considered to play a significant role in the pathogenesis of colitis (Choy, Visvanathan and De Cruz, 2017).

E.Cytokines

The differentiation of original CD4 + T cells, into several distinct types like T-helper 1; T-helper 2; T-helper 17; and T regulatory cell; upon inducement of antigens, each expresses a unique set of cytokines and specific transcription factors and so on, disorders of T cells responses, and T cell subset imbalance, stimulate the high release of cytokines, and chemokine, which result in inflammatory response (Huang and Chen, 2016).

Immune dysregulation in the intestinal mucosa is recognized as the biological mechanism in the progression of ulcerative colitis. In patients with UC, a large number of immune cells (T cells, B cells, dendritic cells and macrophages cells) and cytokines (proinflammatory cytokines such as interferon-γ (IFN-γ), tumour necrosis factor α (TNF-α), interleukin (IL)-6, IL1, IL-17, IL-12, IL-21 and IL-23; anti-inflammatory cytokines such as IL10, IL-35 and transforming growth factor ((TGF)-β) are abnormally expressed in the colon. The imbalance of cytokines modulated by stimulated immune cells is the initial factor that causes diffused superficialinflammatory damage in UC (Loddo and Romano, 2015). (Loddo and

Romano, 2015).

Tumour necrosis factor-α (TNF-α) is a proinflammatory cytokine that has been demonstrated to mediate intestinal tract inflammation as well as, expression of TNF-α increases in colitis (Samaan and Irving, 2016) its role in the promotion of inflammation is performed by the activation of neutrophils, up-regulation of endothelial adhesion molecules responsible for leukocyte migration also promotes activation and infiltration of inflammatory cells and stimulation of hepatic acute phase reactants (Kiesler, Fuss and Strober, 2001).

The T-helper17 cells massively infiltrate the inflamed intestine of IBD patients (Fujino *et al.*, 2003); (Lee, Kwon and Cho, 2018). The studies have suggested that the T-helper17 pathway is of important role in UC inflammation it is now widely accepted that a switch in the T-helper17/Treg balance toward pro-inflammatory T helper17 cells and away from immunoregulatory Tregs is a primary component of immunopathogenesis in colitis immunopathogenesis (Verstockt *et al.*, 2017). Cytokines with anti-inflammatory effects, like IL-4, IL-10, and IL-13 also contribute to the pathogenesis of IBD, decreasing the inflammatory process by down-regulating pro-inflammatory cytokine production (Muzes *et al.*, 2012). Interleukin-4 as an anti-inflammatory cytokine, possesses immunoregulatory effects in UC through the enhancing of the T- helper 2 cell differentiation, and suppression of the excessive response of T-helper- 1 cell (Huang and Chen, 2016).

Also, IL-23, released by macrophages and dendritic cells located in the intestinal mucosa, activates transcription- STAT-4 in memory T lymphocytes, stimulating the production of interferon-γ (IFN-γ). In turn, IFN-γ is responsible for triggering the production of inflammatory cytokines in cells of the innate immune system, contributing to the increase of the inflammation present in colitis (Lee, Kwon and Cho, 2018).

D. Oxidative Stress

Oxidative stress can be explained as a prominent imbalance between oxidants reactive oxygen /nitrogen species (ROS/ RNS) and antioxidant ability in favour of the oxidants, leading to disturbance of redox signalling following by cellular injury, it is observed in many diseases (Sies, 2015);(Hu *et al.*, 2019).

Ulcerative colitis is characterized by massive immune cell infiltrates and showing increasing numbers of poly morphnuclear neutrophils, macrophage, mast cells, and eosinophil (Viveksandeep and Nanda,2015);(Chandrasekar and Venu, 2015) leading to evident production and concentrations of unstable chemical species ROS/RNS significantly contributes to colonic damage and colorectal cancer by modifying the functions of proteins and causing lipid peroxidation (Moura *et al.*, 2015);(Tian, Wang and Zhang, 2017).

Oxidative stress leads to increase membrane permeability and damages of the epithelial layer in the gastrointestinal tract, which in turn results in bacterial invasion, barrier dysfunction, which stimulates the immune response and initiates inflammation (Goyette *et al.*, 2007)(Mao *et al.*, 2019).

Myeloperoxidase (MPO) is an enzyme essentially create in neutrophils and has been used as an effective index of granulocyte cells infiltration in both experimental and human colitis (Prokes, Bendjelloul and Maly, 2000); (Khan, Alsahli and Rahmani, 2018), however, MPO is active in inflamed mucosa in UC patients and participate to the progress of malignancies (Tian, Wang and Zhang, 2017).

F. Adhesion Molecules

Cell adhesion molecule is glycoproteins concerned in the embryogenesis, neoplastic, and inflammatory processes. They also participate in the attachment of leukocytes to endothelial cells in both acute and chronic inflammatory processes (Trzeciak-Jędrzejczyk *et al.*, 2017).

Leukocyte recruitment from the vascular component to the extravascular space is a multistep process that includes rolling, firm adhesion, and transendothelial migration, with the involvement of different families of adhesion molecules in the leukocyte recruitment, including the selectins and their ligands, integrins, immunoglobulins and supergene (Ghosh and Panaccione, 2010).

Selectin, Intracellular adhesion molecule-1 (ICAM-1) and Vascular cell adhesion molecule-1(VCAM-1) are usually expressed at a low basal level (Naito *et al.*, 2006) but their expression can be exaggerated by inflammatory cytokine (TNF-α, IL-1β, and IL-6) which are released from mucosa during intestinal inflammatory (Vainer, 2005).

The selectin or cluster of differentiation 62 (CD62), the function is uniquely limited to the vascular system. It is designated as L-, P-, and E-selectins, representing a family of adhesive receptors expressed on leukocytes (L), platelets and endothelial cells (P) or endothelial cells alone (E). They demonstrated that increased expression of E-selectin on vascular endothelial cells of the colon is characteristic for the active form of IBD (Trzeciak-Jędrzejczyk *et al.*, 2017).

1.3.1.5 Diagnosis

When we consider a diagnosis of UC, clinicians must maintain a broad differential diagnosis such as (anatomic, mucosal infectious, drugs effect, therapeutic effect and functional). Clinicians must obtain a complete and thorough history and inquire about travel, medication history, including antibiotic and nonsteroidal anti-inflammatory drug use, tobacco exposure, and recent hospitalizations (Kaenkumchorn and Wahbeh, 2020).

Infections must be considered and planned out at the time of diagnosis, particularly because they cause the majority of new-onset diarrhea. consequently, C difficile testing must be accomplished (Rubin *et al.*, 2019).

In the patients with UC, C difficile infection is a prevalence of about 3.4 times higher than patients with Crohn's disease,and 8 times that of the general population (Nguyen *et al.*, 2008). Previous antibiotic use (especially fluoroquinolones) and prior hospitalizations increase the danger of C difficile infection. C difficile infection may be present with symptoms mimicking UC, but predominantly it is a "red herring" for the diagnosis of UC. If C difficile infection, it should be treated properly, but the workup for UC Should go on, particularly if symptoms continue. In patients which have a history of travel, one should consider Campylobacter infection (Kaenkumchorn and Wahbeh, 2020).

Also, Cytomegalovirus (CMV) infection must be considered, especially in immune-suppressed patients. CMV gastrointestinal disease is thought to report worse outcomes for patients with known UC (Hendler *et al.*, 2020), although there is continuing debate as to whether CMV contributes pathologically to severe colitis or is a bystander. The prevalence of latent CMV is the same in patients with or without IBD, but the rate of reactivated CMV (with elevated CMV IgM) is higher in patients with IBD (Lv *et al.*, 2017). Up to one-third of patients with acute severe steroid-refractory UC have CMV infection this infection should be evaluated by endoscopy and biopsy15 with hematoxylin and eosin staining, immune histochemistry, or tissue polymerase chain reaction (Sager *et al.*, 2015).

Another condition to consider when patients have symptoms suggestive of UC include malignancy, hemorrhoidal bleeding, rectal prolapse, vasculitis, and irritable bowel syndrome (Magro *et al.*, 2017). Ischemic colitis should be taken into consideration in elderly patients with risk factors such as cardiovascular disease. In younger patients, consider ischemic colitis in patients who have coagulation disorders, medication use, and rarely heavy exercise (Abreu and Harpaz, 2007).

A. Physical Examination

Its assessment for UC extraintestinal manifestations. Toxic appearing patients warrant a more accelerated follow up.

Pallor may be a degree of anaemia. A facial examination must be assessed for scleral icterus, episcleritis, and the buccal mucosa for aphthous ulcers. Patients with colitis may present with a normal abdominal examination or left-sided tenderness. In fulminant disease or toxic megacolon, marked tenderness can be present. The hand examination may reveal digital clubbing. While Skin examination may reveal lesions such as erythema nodosum or pyoderma gangrenosum usually on the lower extremities. The joint examination may refer to the presence of arthritis (Kaenkumchorn and Wahbeh, 2020).

B. Laboratory test

The initial laboratory test should include a complete blood count, electrolytes, blood urea nitrogen, creatinine, liver function tests, iron studies, vitamin D level, C-reactive protein (CRP), erythrocyte sedimentation rate, albumin, and stool testing for microbial infection, particularly for C difficile (Magro *et al.*, 2017).

Three main laboratory abnormalities for patients with UC include iron deficiency anaemia, thrombocytosis, and hypoalbuminemia (Laass, Roggenbuck and Conrad, 2014). Hypoalbuminemia has been associated with higher using corticosteroids, thiopurines, or antitumor necrosis factor agents. Laboratory markers have been studied as an indicator of disease activity and severity. Although the serum C-reactive protein CRP and erythrocyte sedimentation rate are often elevated in severe UC, they may be normal in mild to moderate cases (Solem *et al.*, 2004). About 15% of patients in the general population do not respond to CRP, and UC disease

extent seems to affect sensitivity (Sekar Kathiresan *et al.*, 2006). Furthermore, CRP, is not exclusive to intestinal inflammation and should be considered in systemic inflammatory disease (Nielsen *et al.*, 2000).

Also, Fecal studies such as faecal calprotectin (FC) and stool lactoferrin have been investigated as specific markers of intestinal inflammation. FC and stool lactoferrin are proteins that are liberation into the intestinal lumen as a result of

leukocyte escape to the intestines. Both have shown a perfect correlation with mucosal inflammation seen on endoscopy (Røseth, Schmidt and Fagerhol, 1999).

Several meta-analyses are determined that FC is a good resource to rule out IBD in children who have symptoms related to IBD (Henderson, Anderson and Wilson, 2014). FC show to vary by age in healthy study entrant, the studies represented higher levels in younger patients and decreased to levels of 50 mg/g in age 2 years. (Song *et al.*, 2016).

Also, FC elevation is associated with various inflammatory, infectious, and neoplastic conditions and obesity (MD1 *et al.*, 2015). Specific medications also elevate FC, including non-steroidal anti-inflammatory drugs. Although previous studies are suggested use of proton pump inhibitors may cause an elevation in FC, this was not Consistently (Song *et al.*, 2016).

C. Imaging

Imaging studies are of restricted usefulness in the initial diagnosis of UC. Plain films are more often used to assess for complications of UC rather than in initial diagnosis. A thickened bowel wall may detect thumb printing (Gajendran *et al.*, 2019). The loss of haustration in the colon, or the lead pipe sign, reflects permanent structural colonic change (Sergio *et al.*, 2017). In patients with toxic megacolon, an upright abdominal radiograph is warranted to estimate for colonic dilation, wherein toxic megacolon is defined as a mid transverse colon dilation of more than 5.5 cm.

Imaging of the small bowel is not routinely recommended in patients who have normal-appearing terminal ileum on endoscopy However, in patients with relevant gastrointestinal symptoms not explained by endoscopic findings, suspicion for Crohn's disease, or where proximal disease cannot be safely assessed owing to disease severity,cross-sectional imaging such as computed tomography (CT) scans or magnetic resonance imaging (MRI) or video capsule endoscopy may be ensured (Rubin *et al.*, 2019). An abdominal CT scan is predominantly the initial imaging of choice typically, with both intravenous and oral disparity, given its rapid scan time, broad availability, and high resolution for intra and extra-intestinal pathology. A CT scan permits for the detection of bowel wall abnormalities, such as thickness and abnormal enhancement. A CT scan may be normal in the early stages of the disease because of the inability to detect a subtle mucosal abnormality. In more advanced diseases, wall thickening is increased in the majority of patients. Although the normal colonic wall thickness is 2 to 3 mm, it increases to 8 mm in UC (Kilcoyne, Kaplan and Gee, 2016).

In adults, MRI of the gastrointestinal (GI) tract is immediately an established method when diagnosing UC . The characteristics of MRI include lack of radiation, superior soft-tissue contrast, and multiplanar imaging. restrictions to MRI include cost and longer scan duration. Transabdominal ultrasound examination has emerged as an imaging instrument in UC give the benefits of being noninvasive, less expensive, and well-tolerated. The use of ultrasound examination in Crohn's disease is more -established and its use in UC is gaining interest (Magro *et al.*, 2017). An increased bowel wall thickness of greater than 4 mm is the mean significant variable is seen in UC (Smith *et al.*, 2020).

In compared with endoscopy, ultrasound examination with colour Doppler imaging, have an accuracy of 95% (Pascu *et al.*, 2004). also, Ultrasound examination may be used to describe extraintestinal manifestations such as lymph node enlargement and abscesses. In children, studies prove the usefulness of ultrasound

examination in UC, although a difference of sonographic variables, cutoffs, and scoring systems have been used. The limitations of ultrasound examination are that it is operator subject and it may fail to detect UC in cases of superficial mucosal disease (Kaenkumchorn and Wahbeh, 2020).

D. Endoscopic Evaluation

Colonoscopy remains the cornerstone in UC diagnosis. Nevertheless, depending on endoscopy alone to distinguish IBD from non-IBD colitis is not without limits, warranting additional clinical and histologic details to help in the diagnosis. In ileocolonoscopy at least 2 biopsies from any inflamed area should be done. Additional biopsies from unaffected areas can add more helpful information because as microscopic inflammation can be existing despite the absence of gross findings (Cohen *et al.*, 2008). Doing ileocolonoscopy in the presence of severe disease leads to a higher risk of perforation, therefore sigmoidoscopy with biopsies is a suitable (Rubin *et al.*, 2019).

The macroscopic manifestation of mild UC includes continuous colonic inflammation beginning at the rectum characterized by erythema, loss of normal vascular pattern, granularity. As the severity of the disease increases, erosions, friability, bleeding (which can be spontaneous), and ulcerations are visible, including deep crater-like ulcers in severe UC, which show to be similar to Crohn's disease (Makkar and Shen, 2013).

If ileocolonoscopy manifestation is normal terminal ileum, routine endoscopic assessment of the upper GI tract is not initially required in adults with a new diagnosis of UC, unless upper gastrointestinal symptoms are present (Rubin *et al.*, 2019).

E. Microscopic Evaluation

The microscopic examination must be completed by a pathologist (Rubin *et al.*, 2019). In UC, histologic changes are restricted to the mucosal layer typically in a continuous pattern without skipping. The presence of a granuloma on biopsy should reference a diagnosis of Crohn's disease, although granulomas can also be seen in tuberculosis, sarcoidosis, diversion tissue in UC shows both acute (neutrophilic infiltrates of the crypts, cryptitis) and chronic inflammation features, including basal plasmacytosis, distortion of the crypt architecture, Paneth cell metaplasia, pyloric gland metaplasia, and increased cellularity within the lamina propria (Navaneethan *et al.*, 2012).

The presence of a single feature of chronic inflammation is not diagnostic of UC, Tissue plasmacytosis is possibly the earliest feature related to a diagnosis of UC, appearing in one-third of patients within 2 weeks of the onset of symptoms. In contrast, only 20% of patients have demonstrable crypt deformation within the first 2 weeks, making the distinction of early UC from infectious colitis difficult. Crypt architectural changes appear less commonly in children than adults at presentation. More advanced findings of chronic inflammation show beyond 4 weeks from symptom onset. Histopathologic examination is additionally beneficial to identify dysplasia or neoplasia within the colon.at last, certain histologic features may represent infectious colitis, such as inclusions associated with CMV.

The presence of a granuloma on biopsy should reference a diagnosis of Crohn's disease, although granulomas can also be seen in tuberculosis, sarcoidosis, diversion colitis and other conditions (Cohen *et al.*, 2008). It is important to note that crypt-associated granulomas and giant cells can see in UC and their existence should not mean a change in diagnosis to Crohn's disease (Danese and Fiocchi, 2011).

1.3.1.6 Management

Most UC patients can be treated on an outpatient basis, but hospitalisation is necessary for colitis. The main aim of the treatment of UC is to achieve the greatest possible symptomatic control with the least side effects while allowing the to patient function as normally as possible. The target for treatment increasingly is also appearing at intestinal healing after simple symptomatic control to try and decrease the risk of long term complications and surgery. Treatments can be widely considered as those used to induce remission, such as corticosteroids, 5-aminosalicylic acid (5-ASA) agents, and biologics, these drugs are used for long-term maintenance of remissions such as biologics, 5-ASA agents, and thiopurines (Fell *et al.*, 2016).

A. 5- aminosalicylic acid agents (5-ASA AGENTS)

Sulfasalazine and other 5-ASA agents (eg, mesalazine) is The mainstay of therapy for mild-to-moderate UC. In patients with mild and some with moderate disease, these agents can be effective in inducing remission and also in maintaining remission (Sutherland and MacDonald, 2003).

In general 5-ASA preparations are favoured to sulfasalazine due to a superior side effect profile collective with similar efficacy. However, the absence of a liquid preparation for 5-ASA means that sulfasalazine will often be used in younger children (preschool),. 5-ASA preparations are available as granules, also, for this reason, it is useful for children those unable to swallow tablets such as primary school age (Ford *et al.*, 2011).

The generally benign side-effect profile of 5-ASAs has also resulted in a tendency towards rather higher dosing. The maintenance dose should be like the dose use for induction therapy, although the dose can be reduced after a term of sustained remission. in mild-to-moderate distal UC Topical 5-ASA (suppositories or enemas) are been effective, and usually merging oral and enema therapy is more effective

than either treatment alone for extensive as well as distal UC. The reduction of response to mesalazine within 2–4 weeks is an indication to consider treatment with corticosteroids (Ford *et al.*, 2012).

The major mechanism of salicylate is thought modulates inflammatory mediators derived from both the cyclooxygenase and lipoxygenase pathways (Obi *et al.*, 2016). Moreover, 5- ASA activating the nuclear receptors peroxisome proliferator-activated receptors ligands-γ (PPAR-γ), PPAR-γ is a transcription factor that influence inflammatory responses by inhibiting the production of TNF-α , proliferation and apoptosis of colonic epithelium cells (Criscuoli *et al.*, 2013).

B. Corticosteroids

The majority of patients with moderate-to-severe UC achieved remission on treatment with oral steroids. However, not advised treatment by steroid for maintenance of remission. A single total dose in the morning is suitable to reduce the potential harmful suppression of growth. There are no universally acceptable steroid-tapering protocols (Turner *et al.*, 2012).

Steroid dependency is defined as response or remission with corticosteroid treatment, but the symptom is recurrence when the dose is lowered or within 3 months following complete taper, or if steroids cannot stop within 14–16 weeks. Children with severe UC should be treated with intravenous steroids. Steroid dependency can be avoided by rising maintenance therapy. For distal disease, this typically means a 5-ASA preferred than steroid enema, whereas for more extensive colitis oral beclomethasone dipropionate and a corticosteroid with topical action can be used (Romano *et al.*, 2010).

Corticosteroids bind to the glucocorticoid receptor, and the mechanism of action is based on inhibition of protein transcription and synthesis by affecting the stability of messenger RNA. Corticosteroids downregulate the production of the nuclear factor

kappa B (NF-κB) and many proinflammatory cytokines, such as IL-1, IL-6 and TNF (Silverman and Otley, 2011)**.**

C. Cytotic Agent

-Thiopurines (Azathioprine and 6-Mercaptopurine)

Azathioprine and 6-mercaptopurine (6-MP) are purine analogues generally used in the treatment of steroid-dependent IBD and in children with severe initial presentation of UC. after the start of treatment with thiopurines The therapeutic effect of the drug may take up to 10–14 weeks after the start of treatment, so it is given in a single daily dose.The newest recommendation is to start on the maximum dose of thiopurine with no need to 'increase the dose as was practised historically (Turner *et al.*, 2012).

The mechanism of action of thiopurines is still not clear and it is therefore not entirely known why thiopurines are slow-acting. Incorporation of 6-thioguanine nucleotides (6-TGN) as false purine analogues into DNA and RNA is responsible for the cytotoxic and immunosuppressive effects of thiopurines. After incorporation, DNA damage, cell-cycle arrest and apoptosis occur, resulting in the inhibition of nucleotide and protein synthesis, leading to inhibition of inflammatory gene expression (Coskun *et al.*, 2015)**.**

Common side effects involve headache, rash and GI disturbance. Some patients have an influenza-like illness and pancreatitis occurring in between 1% and 4% of patients. Patients should be au courant of the benefits of treatments and how they are balanced against the side-effect profile, including a small, but increased, risk of malignancy most notably lymphoma (Fell *et al.*, 2016).

D-Calcineurin Inhibitors

1-Cyclosporine

It is a peptide antibiotic its selective inhibition of calcineurin, a regulatory factor involved with the transcription of multiple cytokine genes, inhibits IL-1, IL-2 receptor production and secondarily inhibits T-cell interaction and responsiveness (Douglas and Adrienne,2018). Clinical response to cyclosporine is rapid which can begin after seven days (Calafiore *et al.*, 2013). It can induce colitis remission effectively however long term use is limited due to toxicity and long term failure (Mowat *et al.*, 2011).

Adverse effects include nephrotoxicity, paresthesias, hypertension tremors, seizures, GIT disturbances, and gingival hyperplasia (Sternthal *et al.*, 2008).

2-Tacrolimus

Is an immunosuppressant macrolide antibiotic with calcineurin inhibition ability with action similar to cyclosporine that inhibits IL-2 production and T-cell activation (Jager *et al.*, 2018). Studies demonstrated that tacrolimus is effective short term in the induction of remission for steroid-refractory moderate to severe ulcerative colitis (Magro *et al.*, 2017). The adverse effect includes nephrotoxicity, tremor, diarrhoea, gingival hyperplasia, hirsutism and alopecia (Matsuoka *et al.*, 2018).

E. Biologicals

1- Anti-Tumor Necrosis Factor Agents

The use of monoclonal antibodies (mAbs) is considered to be the last medical treatment option before surgery. However, in severe UC, infliximab can also offer an early treatment option. Monoclonal antibodies targeting TNF cover the majority of the registered therapeutic mAbs used in the treatment of UC (Magro *et al.*, 2017).

Approved therapeutic mAbs for UC is of the immunoglobulin (Ig) G1 antibody type consisting of a Fab fragment and an Fc-portion. Infliximab, an intravenously administered chimeric IgG1 antibody, was the first anti-TNF agent approved for the treatment of UC. The American College of Gastroenterology (ACG) and the European Crohn's and Colitis Organisation (ECCO) recommend the use of infliximab for induction of remission in patients with glucocorticoid-refractory or glucocorticoid-dependent disease (Magro *et al.*, 2017).

Adalimumab (ADL) and golimumab (GLM) are both fully human, subcutaneously administered IgG1 antibodies registered for the treatment of UC. These agents are effective as induction as well as maintenance treatment in UC (Balzola *et al.*, 2012)(Sandborn *et al.*, 2014).

Common adverse effects such as septicemia, tuberculosis, fungal, infection, reactivation of hepatitis B, Listeriosis, less common adverse effects include demyelinating disorders optic neuritis (Park and Jeen, 2015). In addition, all anti-TNF agents have risks of developing antibodies altering their efficacy (Papamichael and Cheifetz, 2017). Hence, checking drug levels and levels of antibodies may allow tailoring of drug dosage or choice of medication to achieve an aclinical response or remission (Papamichael and Cheifetz, 2017);(Katsanos *et al.*, 2019).

2-Anti Adhesion Molecule

The adhesion of leukocytes with the endothelial cells is mediated by integrin, chemokine receptors and endothelial adhesion molecules such as ICAM-1 and VCAM-1 and mucosal addressin cell adhesion molecule -1 (MAdCAM-1) which are overexpressed in UC(Park and van Hemert, 2015).

It is reasonable to consider the use of the gut-selective anti-integrin therapy vedolizumab before the use of systemically acting anti-TNF therapies or small molecules such as Janus kinase inhibitors (JAKI) (Christensen *et al.*, 2018); (Singh *et al.*, 2018) . A study also demonstrated a greater efficacy for vedolizumab compared

with placebo at inducing remission in patients who had previously"failed treatment with"anti-TNF agents (Feagan *et al.*, 2017).

Vedolizumab is a humanized IgG1 mAb that targets α4ß7-integrine, which is present on T cells in the lamina propria. Binding of vedolizumab to α4ß7 prevents binding of α4ß7 to mucosal vascular addressin cell adhesion molecule -1 (MAdCAM-1), which is expressed on endothelial cells, which then blocks the infiltration of α4ß7+ cells into the gastrointestinal mucosa and gut-associated lymphoid tissue, suppressing gut inflammation (Raine, 2014). In contrast to natalizumab (α4-intergrin-inhibitor), vedolizumab (α4 β7 integrin inhibitor) modulates the adaptive immune system without systemic side effect (Krupka and Baumgart, 2014), while, state that not all patients respond effectively to the newer therapeutic options including integrin receptor blockers (Sedda *et al.*, 2018).

3-Janus Kinase (JAK) Inhibitors

Oral JAK inhibitors(Sedda *et al.*, 2018) target one or more of the four cytoplasmic tyrosine kinases (JAK1, JAK2, JAK3, and TYK2) that comprise the JAK family (Cutolo and Meroni, 2013). Janus Kinase (JAK) inhibitors are orally delivered small molecules that target cytokine signalling by preventing phosphorylation of JAK associated with that cytokine activator of transcription of cytokines in the nucleus will be diminished.

The Key cytokines in the pathogenesis of UC are targeted by JAK inhibitors (Kontzias *et al.*, 2012); (De Vries *et al.*, 2017).

Several JAK inhibitors are in developed for the treatment of UC. Tofacitinib inhibiting signalling via all the JAK family members was recently licensed in 2018 for treatment of moderate-to-severe active UC, also it was more effective than placebo in inducing clinical and endoscopic response and remission after 8 weeks of treatment (Balzola *et al.*, 2012); (Panés *et al.*, 2017)(Sandborn *et al.*, 2014). Another study indicates Tofacitinib efficacy in patients with previous anti-TNF failure

The most commonly reported adverse events were diarrhoea, and elevated cholesterol levels (Panés *et al.*, 2017). It is also associated with headache, rash, upper respiratory tract infection, herpes zoster, increased blood creatine phosphokinase, common cold, and elevated liver enzymes (Olivera, Danese and Peyrin-Biroulet, 2017). Less common serious adverse effects included malignancy, serious infection, and primary cases of pulmonary embolism (Fiorino *et al.*, 2018);(Sandborn *et al.*, 2014)

F.Antibiotics Therapy

Since intestinal bacteria play an important role in the development of UC, manipulating the gut microbiota can be achieved by prebiotics, probiotics, and antibiotics (Scott *et al.*, 2015). Prebiotics are dietary compounds that cause specific changes in the composition and/or activity of the gastrointestinal microbiota, but probiotics are live organisms, which when administered in adequate amounts confer a health benefit on the host (Scott *et al.*, 2015). Bio-Three, a commercially available probiotic supplement (Enterococcus T110, C butyricum TO-A, B mesenteric TO-A) produced remission in 45% of patients tested with mild to moderate ulcerative colitis (Shen, Zuo and Mao, 2014).

Antibiotics may influence the course of UC by decreasing concentrations of bacteria in the gut lumen and altering the composition of intestinal microbiota to favour beneficial bacteria"(Scott *et al.*, 2015). The antibiotics should be used in the treatment of UC only if the infection is considered, or immediately before surgery (Dignass *et al.*, 2012). Although controlled trial data are limited, emerging evidence suggests a higher failure rate with metronidazole, suggesting that oral vancomycin should be the first-line agent for the treatment of Clostridium difficile infection in the hospitalized patient with acute severe UC (McDonald *et al.*, 2018). Antibiotics have been used as an adjunct to steroid therapy but have not altered outcomes. However, a systemic review and evidence-based guidelines by the Japanese society of

gastroenterology indicate that antibiotic therapy may induce remission in active ulcerative colitis, however, the selection of the type and duration of antibiotics remains undetermined (Matsuoka *et al.*, 2018).

I. Prebiotics and Probiotics

Prebiotics are non-digestible food ingredients that produce a lower intestinal PH, favouring particular bacterial populations like lactobacillus or Bifidobacterium species, and helping to manage IBD (Tsai *et al.*, 2019). Fructo-oligosaccharide, galacto-oligosaccharide, lactulose, wheat dextrin, psyllium, acacia gum, whole grain corn and inulin are the most commonly used agents as prebiotics (Slavin, 2013).

Probiotics refer to live microorganisms that have shown beneficial effects on the health of the host such as lactobacillus, Bifidobacterium and saccharomyces which are utilized in the treating of Pouchitis their efficacy in the preventing and treatment of UC (Tamaki *et al.*, 2016).

Their mechanism of action has not been confirmed, some studies indicate that probiotics modify membrane permeability and mucosal immune regulation (Zyrek *et al.*, 2007).

1.3.2. Role of Statins IBD

Hydroxymethylglutaryl-CoA reductase inhibitors (statins) are the most commonly prescribed drugs worldwide. Indications for their use are primary and secondary prevention of cardiovascular diseases and treatment of hypercholesterolemia (Peppas *et al.*, 2020). Besides their lipid-lowering effects, statins may exert more complex functions based on their immunomodulatory properties (Peppas *et al.*, 2020). These include inhibition of T-cell activation, antigen-presenting function and leukocyte infiltration of target organs, which make them possible agents for treating immune-related disorders (Ungaro *et al.*, 2016).

Previous studies have reported that statins are associated with reduced use of oral steroids during the acute phase, decreased disease activity index and inflammatory markers in IBD patients (Crockett *et al.*, 2012).

Another study suggested that statins may be associated with a decreased risk of new-onset IBD (Ungaro *et al.*, 2016), raising the possibility that these drugs might also the anti-inflammatory properties which suggest that they can regulate molecules important for immunomodulation. statin was found to inhibit the production of TNF- and inducible nitric oxide synthetase by microglia and astrocytes. In addition demonstrated that atorvastatin treatment could either inhibit or reverse chronic and relapsing experimental autoimmune encephalomyelitis, the archetypal model for multiple sclerosis in vivo (Youssef *et al.*, 2002).

These observations suggest that statins may be beneficial in multiple sclerosis and other autoimmune diseases. Here we examine the immunomodulatory effects of atorvastatin in acute and chronic experimental colitis induced by either acetic acid dextran, sulfate sodium or trinitrobenzene sulfonic acid (Park *et al.*, 2004).

1.3.2.1. Structure

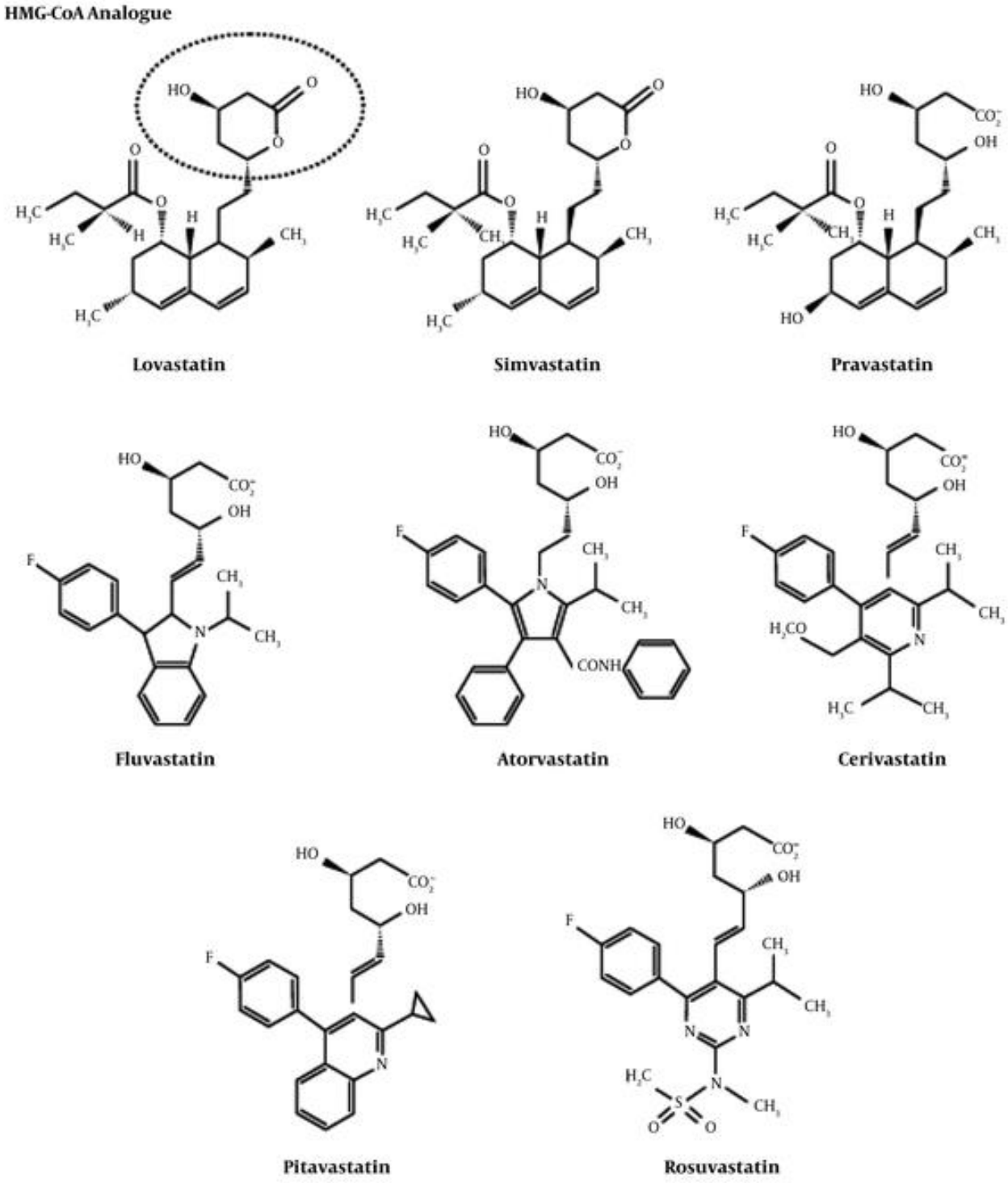
The chemical structure of all statins consists of the pharmacophore and its moiety containing a ring system with different substituents. The pharmacophore is shared among all statins, it is a dihydroxyheptanoic acid segment that is very similar to the HMGCoA substrate. The ring system consists of a complex hydrophobic structure covalently linked to the pharmacophore and it is involved in binding interactions with the HMG-CoA reductase enzyme (Egom, And and Hafeez, 2016).

It has also been shown that the HMG-CoA reductase is stereoselective and as a result, all statins need to have the required stereochemistry of chiral carbon atoms, on their pharmacophore (Roche, 2005). The statin pharmacophore inhibits the HMG-CoA reductase enzyme and it binds to the same active site as the HMG-CoA substrate naturally would (Egom, And and Hafeez, 2016).

Statins exist in two forms, lactone (inactive) and open-ring hydroxy acid (active) forms. The HMG-like moiety that all statins share, may present in the inactive form as a lactone. Simvastatin and lovastatin are administered as lactone prodrugs and subsequently transformed into active metabolites. The remaining statins are formulated in the pharmacologically active form as a β-hydroxy acid. In vivo, lactone prodrugs are enzymatically hydrolyzed to their hydroxy acid pharmacophores in the liver to achieve pharmacological activity (Egom, And and Hafeez, 2016). On the other hand, the remaining statins, in β-hydroxy acid form are already in their active form and possess two hydroxyl groups in an alkyl chain at the β and δ positions for the carboxylic acid group (Sirén, 2017).

Statin drugs are also divided into two groups: naturally or fungal-derived (type 1) and synthetic (type 2) (Schachter, 2005). Type 1 statins include simvastatin, pravastatin, and lovastatin. Type 1 statins contain a substituted decalin ring structure that resembles the first-ever statin discovered, mevastatin (also known as compactin). Type 2 statins are those that are fully synthetic and with larger, fluorophenyl, groups linked to the HMG-like moiety. These include fluvastatin, atorvastatin, rosuvastatin, and cerivastatin (Sirén, 2017).

One of the main differences between the type 1 and type 2 statins is the replacement of the fluorophenyl group of type 2 statins with the butyryl group in type 1 statins. These specific groups are responsible for additional polar interactions that cause tighter binding to the HMGR enzyme. Functionally, the methyl ethyl group attached to the central ring of the type 2 statins replaces the decalin of the type 1 statins. The butyryl group of the type 1 statins occupies a region similar to the fluorophenyl group present in the type 2 inhibitors (Egom, And and Hafeez, 2016).



Figure(1.1) Structures of Statins

1.3.2.2.Types of Statines

1. atorvastatin (Lipitor, Torvast)
2. fluvastatin (Lescol)
3. lovastatin (Mevacor, Altocor, Altoprev)
4. pitavastatin (Livalo, Pitava)
5. pravastatin (Pravachol, Selektine)
6. rosuvastatin (Crestor)
7. simvastatin (Lipex, Zocor)

Some combination medications also contain statins. Among them are:

1. amlodipine/atorvastatin (Caduet)
2. ezetimibe/simvastatin (Vytorin)

1.3.2.3 Mechanism of Action

Statins work by competitively blocking the active site of the first and key rate-limiting enzyme in the mevalonate pathway, HMG-CoA reductase. Inhibition of this site prevents substrate access, thereby blocking the conversion of HMG-CoA to mevalonic acid. Within the liver, this reduces synthesis, leading to increased production of microsomal HMG-CoA reductase and increased cell surface LDL receptor expression. This facilitates increased clearance of LDL-c from the bloodstream and a subsequent reduction in circulating LDL-c levels by 20% to 55%.12(Ward, Watts and Eckel, 2019). In addition to reducing LDL-c and cardiovascular morbidity and mortality, statins may have additional non–lipid-related pleiotropic effects. These include improvements in endothelial function, stabilization of atherosclerotic plaques, anti-inflammatory immunomodulatory and antithrombotic effects, effects on bone metabolism, and reduced risk of dementia. These additional benefits are primarily thought to arise because of inhibition of the synthesis of isoprenoid intermediates of the mevalonate pathway (Buhaescu and Izzedine, 2007).

1.3.2.4 Indication

All statins reduce serum LDL in a non-linear, dose-dependent fashion, but differ in their absorption, excretion and solubility. Statins target hepatocytes through inhibition of HMG-CoA reductase, a key regulator of cholesterol biosynthesis This reduction in intracellular cholesterol production results in upregulation of hepatic LDL receptors which in turn reduces levels of circulating LDL. The downstream effect is reduced accumulation of oxidized LDL within the arterial intima and thwarted inflammatory cascade which promotes monocyte recruitment and foam cell formation, the initial and key step in atherogenesis. (Almeida and Budoff, 2019). However even more fascinating are statin's pleiotropic effects, cholesterol-independent CV protective benefits as a result of inhabiting the production of intermediates in the cholesterol biosynthetic pathway(figure1.2) (Oesterle, Laufs and Liao, 2017).

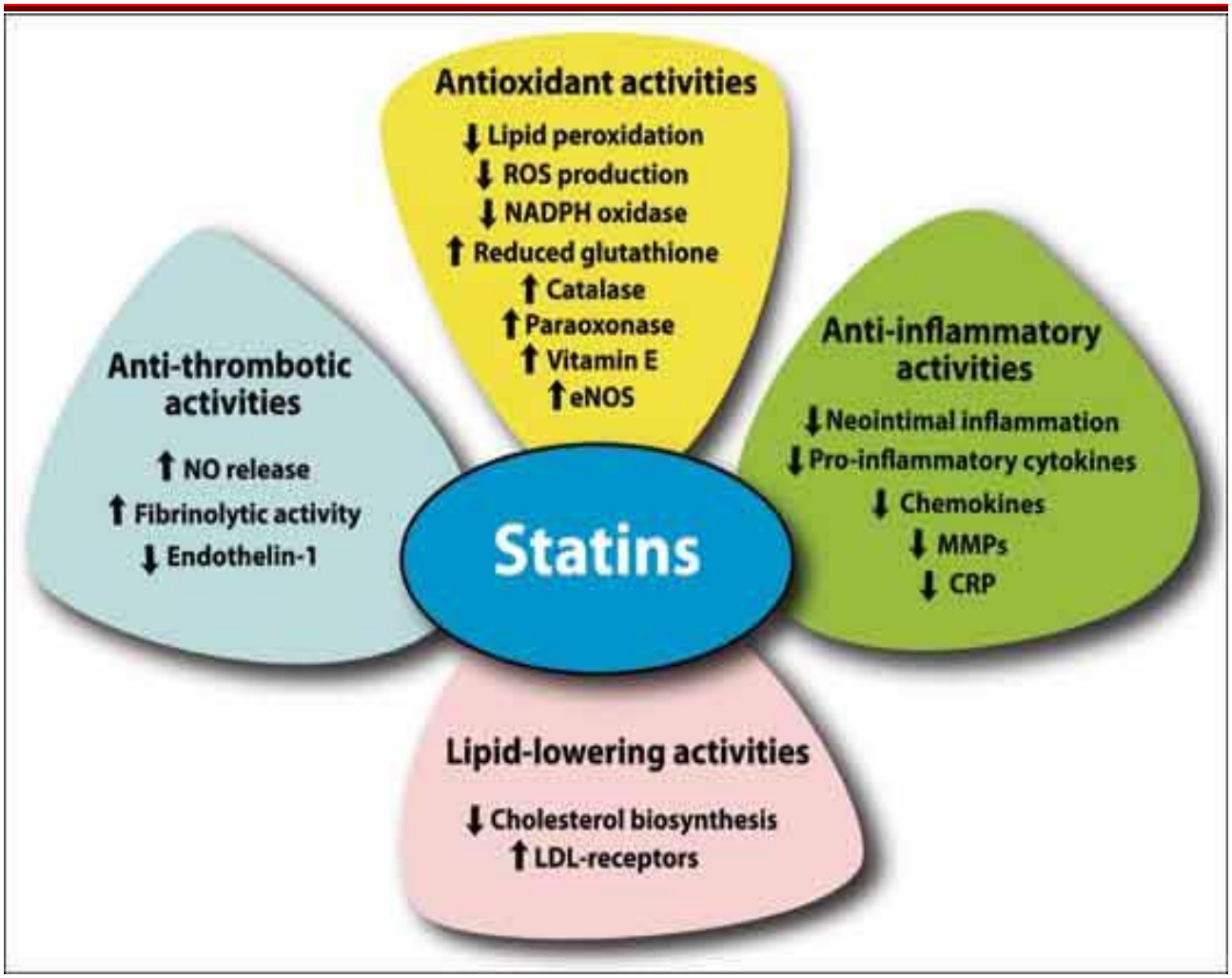


Figure (1.2): Indication of Statins

Statins have been shown to have a beneficial effect on vascular tone. In cell studies, HMG-CoA reductase inhibition resulted in upregulation of endothelial nitric oxide (NO) synthase activity, resulting in increased bioavailability of NO, an important regulator of vascular tone, platelet aggregation and vascular smooth muscle cell SMC proliferation (Laufs *et al.*, 1998).

The latter is a key driver of atherosclerotic plaque progression and statin medications have been shown to reduce proliferation and migration of vascular SMCs.

This process is especially evident in the cardiac transplant population .when the patients treated with pravastatin compared with patients receiving no HMG-CoA reductase inhibitor had lower rates of coronary artery vasculopathy .the pathogenesis

of which relates to activation of smooth muscle cells as a result of a chronic immune response in the transplant recipient (Almeida and Budoff, 2019).

Statin medications have also been implicated in reducing platelet aggregation as well as having antithrombotic properties that may contribute to the overall reduction in cardiovascular death (Sikora *et al.*, 2013). Another important pleiotropic effect of HMG-CoA reductase inhibitors is the anti-inflammatory properties and reduction in oxidative stress. In vitro and in vivo models, as well as evidence from clinical studies, have supported the idea that statins reduce systemic inflammation (Diamantis *et al.*, 2017).

Statins reduce CRP levels as well as inhibit mediators of inflammation such as tumour necrosis factor-alpha and interleukins. These anti-inflammatory effects are critical in curbing the progression of atherosclerotic plaque (Singh *et al.*, 2016). also Plaque regression involves removal of lipid and necrotic core, restored endothelial function and cessation of intravascular smooth muscle cell proliferation (Almeida and Budoff, 2019).

1.3.2.5 Side Effect of Statins

A. Musculoskeletal

Nearly all of the statin drugs are associated with musculoskeletal side effects. Myalgia is the most common symptom, and myositis is less common and associated with a rise in creatine kinase (CK). Rhabdomyolysis is the most severe musculoskeletal form observed, with a rise in CK greater than 10x the upper limit of normal with associated features including myoglobinuria, renal impairment and serum electrolyte abnormalities(Ramkumar et al., 2016).

The exact mechanism of statin-related myopathy is unknown, though it involves a complex interplay of drug, patient-related factors and concomitant therapy. Statins have been associated with mitochondrial dysfunction associated with a reduction in

coenzyme Q10 levels(Bitzur *et al.*, 2013); (Stroes *et al.*, 2015). Another mechanism is the lowering of farnesyl pyrophosphate and geranylgeranyl pyrophosphate, which are end products of the mevalonate pathway and thus involved in the maintenance of cell growth (Bitzur *et al.*, 2013).

Statins have also been shown to alter cholesterol content in skeletal muscle cells which alter the flow of ion channels including calcium, making them vulnerable to cell injury and death (Bitzur *et al.*, 2013).

B. Hepatic Dysfunction

Statin therapy has been associated with elevated hepatic transaminases in up to 1-3% of patients(Russo *et al.*, 2014). This usually is dose-dependent and occurs within the first three months of commencing therapy, and is not usually associated with any long-term hepatic dysfunction. There also appears to be no significant differences amongst the different statin drugs with regards to rates of hepatotoxicity (Russo *et al.*, 2014).

However, these are sufficiently uncommon that overall the incidence of hepatic failure in patients taking statins appears to be no different from the incidence in the general population(Egom, And and Hafeez, 2016). Thus, when serious hepatotoxicity is encountered in a statin-treated patient, undiagnosed, and nonstatinrelated liver diseases should be strongly considered in the differential diagnosis (Mancini *et al.*, 2011). The pattern of more severe hepatotoxicity attributed to statins has included hepatocellular, cholestatic, and autoimmune injury (Russo *et al.*, 2014).

The most commonly reported hepatic adverse effect is the phenomenon known as “transaminitis” in which liver enzyme levels are elevated in the absence of histopathological changes. Although the underlying mechanism remains unclear, it may result from altered lipid components within the hepatocyte membrane, leading to increased permeability and subsequent “leakage” of liver enzymes (Calderon *et al.*, 2010).

C. Diabetes Mellitus

Statins have been shown to increase the risk of diabetes mellitus in that they can disrupt insulin signalling pathways, affect pancreatic beta-cell function and may contribute to increased insulin resistance (Bang and PeterM.Okin, 2014);(Cederberg *et al.*, 2015). How statins increase the risk of DM is not clear, but the lower cholesterol levels produced by statins may contribute to the effect. High serum cholesterol levels are associated with a reduced risk of DM. Changes in cellular cholesterol content could impair insulin secretion by disrupting voltage-gated calcium-channel function in pancreatic beta-cell, thereby reducing the fusion of insulin granules with the cell membrane for subsequent export. Alternatively, statins could reduce peripheral insulin sensitivity or glucose metabolism by reducing myocyte mitochondrial function or affecting other aspects of muscle metabolism (Ramkumar *et al.*, 2016).

D. Renal

Statins can influence the kidney in two main pathways. Rhabdomyolysis can induce tubular obstruction causing tubular injury and ischaemia. Statin therapy can be associated with benign proteinuria due to inhibition of the tubular reabsorption of small molecular weight proteins. The clinical significance of this mild proteinuria is unknown, as the protein differs from that of other glomerular diseases. There has been no evidence of long-term renal dysfunction from statin therapy (Brown, 2008).

E. Possible Adverse Effects of Statins on Cognition

Neurological conditions that have been associated with statin use include hemorrhagic stroke, cognitive decline, peripheral neuropathy, depression, confusion/memory loss and aggression, and personality changes. Lipophilic statins

are thought to have a higher risk because of their increased ability to cross the blood-brain barrier (Ward, Watts and Eckel, 2019);(Fong, 2014).

Early research suggested that lowering cholesterol concentrations may be associated with an increase in violent or suicidal deaths.

Other studies on the Biochemistry of Statins showed that both chronically low and medically lowered serum cholesterol may be associated with an increased incidence of depression.

Although concerns have been raised, statins do not appear to be associated with an increased risk of suicide or depression (Egom, And and Hafeez, 2016).

2.1. Materials

2.1.1: Instruments and Equipment:

Table 2.1 lists the instruments and equipment, which were utilized in the study with their suppliers.

Table 2.1.1 List of instruments and equipment

NO.	Instrument / equipment	Company	Country
1	High speed cold centrifuge	Eppendrof	Germany
2	Micropipettes	Eppendrof	Germany
3	Eppendrof tube	Sigma	England
4	Sensetive balance	Sartorius	India
5	Refrigerator	Concord	Lebanon
6	Centerifuge	Hettich	Germany
7	Incubater	Memmert	Germany
8	Elisa reader	Biotek	England
9	Elisa washer	Biotek	England
10	Feeding tube size 6	Angeltouch	India

2.1.2: Kits:

Table 2.2.2 lists of the kits that were used in the study with their companies and countries of origin

Table 2.1.2: List of kit

No.	Kit	Company	Country
1	MPO	Elabscieenc	China
2	IL6	Elabscieenc	China
3	TNF-a	Elabscieenc	China
4	CRP	Elabscieenc	China

2.1.3: Chemicals&Drugs :

Table 2.1.3 lists the chemical and biological materials used in this work are listed in Table 2.1.3 :

Table 2.1.3: List of Chemical materials with their remarks

NO.	Chemicals & Drugs	Company	Country
1	Chloroform	BDH	England
2	Acetic Acid	BDH	England
3	Formaline	BDH Chemical Ltd	England
4	Distilled Water	SDA	Iraq
5	Rosuvastatin	Pioneer	Iraq
6	Atorvastatine	Astrazeneca	England
7	Xolone	EEPI	Egypt

2.2.Methods

2.2.1.Experimental animals

All experimental procedures utilizing mice were following National Institute of Health guidelines.

This study was conducted on 84 adult male albino –Wister mice weighting (20–25 g) were obtained from the Experimental Animal Center (Baghdad, Iraq). They were maintained in our animal house in) standard plastic cages controlled at a constant temperature of 23±2 °C with a relative humidity of 55±5% and a 12 h light/dark cycle((Niu *et al.*, 2013) and the air of the room was changed continuously by using ventilating vacuum and supplied with fresh water and standard chow food (ad libitum). The mice were allowed one week to acclimate to the animal house environment before the commencement of our experiment The animals were allowed free access to tap water and standard laboratory chow.

2.2.2. Preparation of animals

Mice were starved for at least 24hrs before the induction of colitis but were be allowed free access to tap water. During starvation, the mice were distributed randomly kept in cages provided with a wide wire–mesh floor to avoid coprophagy. On the day of the experiment, water was held two hours before the procedure .

2.2.3 Preparation of drugs

All drugs were freshly prepared before administration on the day of the experiment. Investigated drug atorvastatin, rosuvastatin and standard prednisolone were suspended in 25ml distilled water.atorvastatin was used at two doses 5mg/kg and 50 mg/kg (this dose was chosen depending on previous studies that have been showing an improved clinical outcome in animal models of murine colitis, experimental

autoimmune encephalomyelitis, arthritis, and systemic lupus erythematosus (SLE)). These beneficial effects are partly attributed to statins immunomodulatory role in suppressing Th1 and Th17 responses, while promoting Th2 responses and reducing the levels of pro-inflammatory cytokines IL-1β, TNF-α, and IL-6 (Lei *et al.*, 2016) (Aktunc *et al.*, 2011).

On the other hand rosuvastatin dose at 2mg/kg and10mg/kg was selected according to other studies reporting cytokines suppression effect of (Naito *et al.*, 2006)

(Maheshwari *et al.*, 2015).

Prednisolone was used as standard therapy in a dose of 1mg/kg (Melgar *et al.*, 2007).

All drugs will be given orally for 10days.(3days before induction and 7days after induction)(Lei *et al.*, 2016).

2.2.4.Induction of colitis

The acetic acid Induced colitis is a widely used in animal model of colitis((Küpeli Akkol *et al.*, 2020).

Briefly, 24 h fasted mice were lightly anaesthetized with ether. A 6 inch catheter was then carefully inserted into the colon and the tip was 4 cm proximal to the anus. To induce colitis, a solution of 2 ml of acetic acid (4%, v/v) in 0.9% saline was instilled into the lumen of the colon and maintained in a supine Trendelenburg position for 30 s to prevent the leakage of the intracolonic instil. In normal control experiments, mice received 0.9% saline alone using the same technique((Niu *et al.*, 2013).

2.2.5. Experimental protocol

Animals were divided into seven groups, each group consisted of ten animals (n=14 /group except control group=10) as follows:

Group I: served as a normal control group, was received normal saline (5ml/kg) rectally.

Group II: served as a colitis control group, was received only 4% v/v acetic acid (5ml/kg) rectally.

Group III: served as rosuvastatin group, were received acetic acid rectally + (2mg/kg) orally as low dose.

Group IV: served as rosuvastatin group, were received acetic acid rectally + as rosuvastatin (10mg /kg) orally. as high dose.

Group V: served as atorvastatin group, were received acetic acid rectally + atorvastatin (5mg /kg) orally.

Group VI: served as atorvastatin group, were received acetic acid rectally + atorvastatin (5mg/kg) orally.

Group VII: served as the positive control group, were received acetic acid rectally + prednisolone as standard therapy (1mg /kg) orally.

All these drugs were given orally one hour following the induction of colitis for ten days(3day before induction and7 days after induction) by using oral gavage.

After the end of the experiment, animals were sacrificed after 24 hrs of starvation by an overdose of chloroform inhalation and then the abdomen was rapidly dissected and open and the colon was removed.

The pieces of colons were cut open in an ice bath cleansed gently using normal saline, and observed normally for macroscopic and microscopic assessment.
Then samples were cut into two pieces, the first one-piece was used for histopathologic assessment (maintained in neutral formalin 10% as a fixator)and the second one-piece for biochemical study.

2.2.6 . Assessment of colitis severity

2.2.6.1.Clinical evaluation (Gross morphological changes)

A- The Disease activity index defined(DAI)

Was used to assess the disease clinically which involve bodyweight reduction {0=weight gain or no reduction, 1= 1-5 % reduction, 2=6- 10% reduction, 3=11-15 % reduction, 4=more than 15% reduction}; the consistency of faeces {0=normal, 2=loose faeces, 4=diarrhea}; and bleeding of rectum {0= normal, 2= mild bleeding, 4=severe bleeding}. The calculation was made by the combination of the total scores of DIA.

The normal stool was well-formed pellets; loose stool was pasty stools that didn't stick to the anus; diarrhoea was liquid stools that stick to the anus ((GONG1 *et al.*, 2019).

B. Colon Oedema

Assessment of Colon Weight-to-Length Ratio.

oedema was determined by measuring the colon weight by a sensitive balance and length by using Ruler, Mice were dissected and their colons were extirpated, opened longitudinally, and rinsed gently under running water to remove the faeces.

Colons were placed on non-absorbent surfaces and weight-to-length ratios were blindly assessed. Extirpated colons were stored at –80°C for biochemical analyses. ((Manna, Abu-Raghif and Abbood, 2017) (Owusu *et al.*, 2020).

C-The Macroscopic Colonic Score(MAC)

MAC was determined visually by the scoring system described as follows: the score is assigned based on the clinical features of the colon using 5 point scale ranging from 0to6 follows:

0= absence the signs of inflammation;

1= redness or swelling;

2= swelling and redness;

3= linear ulceration with minimal inflammation;

4= two or more sites of ulceration with prominent inflammation; extending for more than 1cm along the colon.

5= initial necrosis;

6= severe necrosis

(Gupta, Motiwala and Mahajan, 2018)(Amirshahrokhi, Bohlooli and Chinifroush, 2011).

2.2.6.2.HistoPathological Evaluation(Microscopic scoring)

The colon after the macroscopic evaluation of the mucosa was divided into two pieces for pathologic evaluation and biochemical studies. The colonic samples were fixed in 10% formalin, Analysis was according to ((Manna, Abu-Raghif and Abbood, 2017). and dehydrated, embedded in paraffin, deparaffinized with xylene, cut into 4 µm sections and stained by Hematoxylin and eosin (H&E). Slides were examined and scored for histopathological evaluation. The slides were coded to prevent observer bias during evaluation. All tissue sections were examined in a blinded fashion by experienced histopathologists and results were scored according to (GAUDIO *et al.*, 1999).

Table 2.1.4 .Histopathological score of colitis (GAUDIO *et al.*, 1999)

Score	Destruction of epithelium and/ or glandular crypts	Dilation of glandular crypts	Depletion and loss of goblet cells	Inflammatory cell infiltration	Edema	Hemorrhagic mucosa	Crypt abscess	Apoptosis	Dysplasia
0	morphologically normal	Normal aspect	Normal aspect	absence of infiltration	Absent	Absent	Absent	absent	absent
1	focal destruction	Focal dilation	slightly depleted goblet cells	infiltrate at the sub- epithelial and lamina propria level or crypt bases infiltration	Focal	Focal	focal	Focal	Focal
2	zonal destruction	Zonal dilation	zonal or moderately depleted goblet cells	infiltration reaching muscularis mucosa	zonal and/or moderately diffuse	Zonal	Zonal	Zonal	Zonal
3	diffuse and/or mucosal ulceration involving submucosa and/or diffuse crypt loss	diffusely dilated crypts	diffusely or complete depletion of goblet cells	severe and extensive infiltration reaching submucosa and/or involving muscularispropria	extensive and severe	Diffuse	Diffuse	Diffuse	diffuse

2.2.6.3.Biochemical Evaluation :

A: C- Reactive Protein(CRP)

C-reactive protein (CRP) is recognized as one of the most important proteins in the acute inflammation phase. CRP may not be used alone as a diagnostic or treatment

biomarker in CD and UC because it has insufficient functional characteristics to act as a substitute for clinical endoscopic or radiographic findings. CRP is a noninvasive adjunctive index that can be used in both CD and UC to direct the evaluation by further endoscopy or radiography. ((Norouzinia *et al.*, 2017) The serum CRP was measured using an enzyme-linked immunosorbent assay **(**ELISA) kit according to the manufacturer's instructions.

B: Tumour Necrosis Factor-alpha (TNF-α)

TNF-α is a pleiotropic pro-inflammatory cytokine implicated in a wide range of cellular processes including cell proliferation, survival and death. In addition, TNF-α signalling is associated with the regulation of several inflammatory pathways including the cyclooxygenase-2 (COX-2) and inducible nitric oxide synthase (iNOS) pathways. Hence, TNF-α is a key mediator in the inflammatory response. TNF-α is predominantly secreted by monocytes, macrophages and natural killer cells ((Gareb *et al.*, 2020).

The tissue TNF-a were measured using an enzyme-linked immunosorbent assay (ELISA) kit according to the manufacturer's instructions(Xiao *et al.*, 2016).

C: Interleukin-6(IL-6)

IL-6 is produced by various cell types. the previous study has demonstrated that IL-6 signalling plays a key role in the pathogenesis of IBD.

Elevated levels of IL-6 are found in both the blood and colonic mucosa of IBD patients compared to those of healthy controls ((Ye *et al.*, 2020).

The tissue TNF-a were measured using an enzyme-linked immunosorbent assay (ELISA) kit according to the manufacturer's instructions(Xiao *et al.*, 2016).

.-Tissue Homogenization:

1. Cut a piece of tissue using the razor blade.

2. Weigh (0.1 gm) of the piece of the tissue and then place the sample on the ice again to keep it from getting warm.

3. Working on ice, chop the sample tissue into small pieces with scissors or a single-edge razor blade. The tissues must stay cold.

4. Add (3 ml) of phosphate buffer (pH=7.2).

5. Homogenize on ice by grinding with a pestle for several minutes until no more chunks are visible.

6. Transfer the sample in aliquots to Eppendorf tubes and centrifuge in the cold room at 14,000 x g for 10 min. Carefully collect the supernatant.

- ELISA procedure:

1. Add 100µL each dilution of standard, blank and sample into the appropriate well. Cover the plate with the sealer provided in the kit. Incubate for 90 min at 37°C.
2. Decant the solution from each well, do not wash. Immediately add 100µL of Biotinylated Detection Ab working solution to each well. Cover with the Plate sealer. Gently mix up. Incubate for 1 hour at 37°C.

3. Decant the solution from each well, add 350µL of wash buffer to each well. Soak for 1-2 minutes and aspirate or decant the solution from each well and pat it dry against clean absorbent paper. Repeat this wash step. 3 times in total.
4. Add 100µL of HRP Conjugate working solution to each well. Cover with the Plate sealer. Incubate for 30 minutes at 37°C
5. Decant the solution from each well, repeat the wash process five times as conducted in step 3.
6. Add 90µL of Substrate Reagent to each well. Cover with a new plate sealer. Incubate for about 15 minutes at 37°C. Protect the plate from light.

7. Add 50 µL of Stop Solution to each well. Adding the stop solution should be done in the 50uL of Stop Solution to each well.
8. Determine the optical density (OD value) of each well at once, using a microplate reader set to 450nm.

D: Myeloperoxidase as a pro-inflammatory marker(MPO)

MPO is an enzyme found in high content in neutrophils, where it is used for converting hydrogen peroxide (H2O2) to hypochlorous acid (HOCl). HOCl is a reactive oxygen species that chronically damage biomolecules, making it cytotoxic both to pathogenic cells such as bacteria and normal tissue cells. MPO could be a good measure of neutrophils.

Neutrophils quickly migrate from the bloodstream to sites of inflammation, often in response to a bacterial infection. Therefore, the number of leukocytes in a tissue can be used as an indication of the severity of inflammation.

For this project, MPO levels are measured and used as an approximation of leukocyte count and therefore degree of inflammation(Ahl, 2010).

-Procedure assay:

10 µl of the serum was mixed with 220 µl of 50 mM phosphate buffer, pH 6.0, and containing O-dianisidine dihydrochloride. The reaction was started by adding 40 µl of 1% Hydrogen peroxide. The reaction was stopped by adding 50 µl of sodium azide. The change in absorbance was measured at 450 nm for three minutes(Murray *et al.*, 2020).

2.3.Statistical analysis

Statistical package for social science version (SPSS)23 software program was used to summarize, analyze and present the data. Quantitative (numeric) variables were expressed as mean and standard deviation. One-way analysis of variance (ANOVA) was used to study difference in mean of quantitative variables among groups; then followed by post hoc least significant difference (LSD) test to evaluate mean difference within groups. P value less than 0.05 were considered significant(Daniel,2009)

3.Results

3.1. Effect of acetic acids on colonic mucosa in mice

Rectally administration of 5 ml /Kg of (4 % acetic acid) in mice result in extensive ulceration and necrosis in animal (100 % induction) compared with normal colonic mucosa of healthy groups (apparently healthy group).

3.1.1. Clinical parameter

Diseases activity index (DAI) of colonic injury in experimental induced colitis was highly significant elevated compared to normal control group as shown **table 3-1** .On the other hand the mean colonic odema is significantly higher in colitis group versus in control normal group as shown in **tables 3-1** . Colitis group also exhibited a highly significant increase in macroscopic scores of colonic mucosal injury as compared with control group as shown in **table 3-1** and figure **3-1 A, B .**

3.1.2. Effect of acetic acid on microscopical score

Histopathological score reflective of colonic injury in experimentally induced colitis was shown to be highly significant increased in colitis group as compared with healthy control group as shown in table 3.1

3.1.3. Effect of acetic acid on C-reactve protin (CRP)

CRP was highly significant increased in colitis group as compared with in healthy control group as shown in table 3.1

3.1.4. Effect of acetic acid on score of cytokines (TNF-α and IL-6)TNF-α also IL-6 were highly significant increase in colitis group as compared with in normal control group as shown in **table 3-1**.

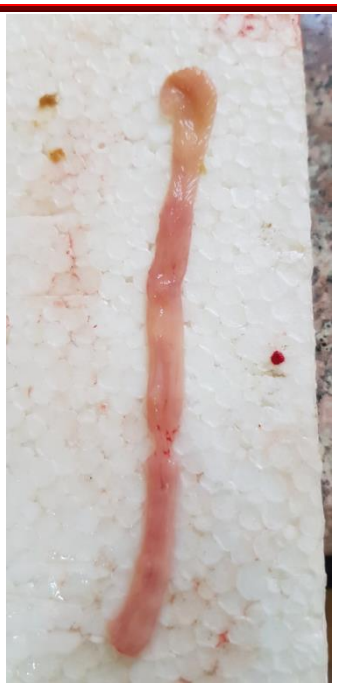


Figure (3.1) : Gross features of normal colon showing shiny serosa and healthy mucosa

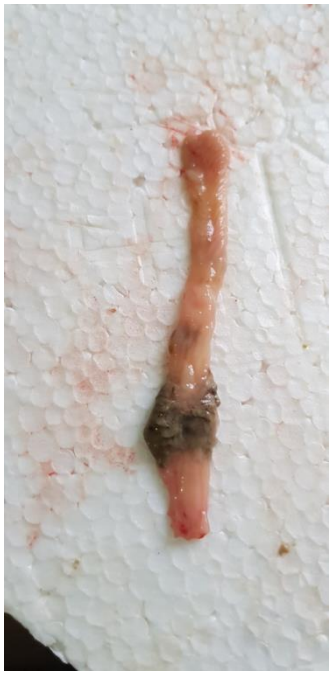


Figure (3.2): colitis with marked edema, congestion, ulceration and necrosis

3.1.5 .Effect of acetic acid on score of oxidative stress (MPO)

expression scores of oxidative stress markers MPO was highly significant increased in colitis group compared to in healthy control group as shown in **table 3-1**.

Table 3-1. Comparison of Clinical, microscopic score, and oxidative stress parameters in healthy control and acetic acid induced colitis groups

Variable	Acetic acid induce colitis group	Healthy control group	*p value*
DIA	9.89 ± 0.5	0.00 ± 0.00	<0.01**
W / L	0.21 ± 0.02	0. 9 ± 0.04	<0.01**
MAC Score	3.20 ± 0.4	0.00 ± 0.00	<0.01**
HISTO Score	9.60 ± 2.9	0.00 ± 0.00	<0.01**
IL-6	79.15 ± 7	27 ± 2	<0.01**
TNF-α	790 ± 73	299 ± 20	<0.01**
MPO	100 ± 14	30 ± 8	<0.01**
CRP	8.05 ± 0.3	2.4 ± 0.2	<0.01**

3.2. Effect of rosuvastatin low dose on acetic acid –induced colitis in mice

3.2.1. clinical parameters

Low dose rosuvastatin treatment exhibited a significant decreased in colon weight/ colon length ratio as compared with in colitis group .

Disease activity index (DAI) of colonic injury was significant decreased with low dose rosuvastatin treatment as compared with in colitis group .

Low dose Rosuvastatin also significant decreases macroscopic score of colonic mucosal injury as compared with in colitis group.all that shown in **table 3.2**

. 3.2.2. Effect of low dose rosuvastatin on microscopical score

Histopathological score reflective of colonic injury was highly significant decreased with low dose rosuvastatin treatment compared with in colitis group as shown in **table 3.2**

3.2.3. Effect of low dose rosuvastatin on C-reactve protin (CRP)
CRP was highly significant decreased in rosuvastatin(low dose) group as compared with in colitis group show in **table 3.2**

3.2.4 .Effect of rosuvastatin(low dose) on cytokines (TNF-α and IL6).
Low dose rosuvastatin treatment was significant decrease expression scores of(IL-6 and TNF-a) as compared to in colitis group as shown in **table 3.2**

3.2.5 .Effect of rosuvastatin(low dose) on oxidative stress (MPO)

expression scores of MPO was significant decreased with low dose rosuvastatin treatment as compared with in colitis group as shown in **table 3.2**

Table 3-2. Comparison of Clinical, microscopic score, and oxidative stress parameters in Acetic acid induce colitis group and low dose rosuvastatin group.

Variable	Acetic acid induce colitis group	low dose rosuvastatin group	*P value*
DIA	9.89 ± 0.5	4.12 ± 0.4	<0.01**
W\L	0.21 ± 0.02	0.16 ± 0.01	<0.01**
Macro Score	3.20 ± 0.0	2.60 ± 0.5	<0.05*
HISTO Score	9.60 ± 2.9	7.80 ± 1.4	<0.05*
IL-6	79.15 ± 7	58 ± 1	<0.01**
TNF-α	790 ± 73	640 ± 36	<0.05*
MPO	100 ± 14	78 ± 6	<0.05*
CRP	8 ± 0.3	403 ± 0.4	<0.01**

3.3. Effect of rosuvastatin(high dose) on acetic acid –induced colitis in mice

3.3.1. clinical parameters

High dose rosuvastatin treatment was more significant than low dose decreased in colon weight/ colon length ratio as compared with in colitis group as shown in **table 3-3.**

Disease activity index (DAI) of colonic injury was highly significant decreased with high dose rosuvastatin treatment in colitis group as shown in **table 3.3**

High dose Rosuvastatin also more significant than low dose in decrease macroscopic score of colonic mucosal injury as compared with in colitis group as shown in **table 3.3**.

3.3.2. Effect of high dose rosuvastatin on microscopical score

Histopathological score reflective of colonic injury was highly significant decreased with high dose rosuvastatin treatment compared with in colitis group as shown in **table 3.3**

3.3.3 Effect of high dose rosuvastatin on C-reactve protin (CRP)

CRP was highly significant decreased in rosuvastatin(high dose) group as compared with in colitis group as show in **table 3.3**

3.3.4 .Effect of rosuvastatin(high dose) on cytokines (TNF-α and IL6)

High dose rosuvastatin treatment was more significant decrease expression scores of(IL-6 and TNF-a)than low dose as compared to in colitis group as shown in **table 3.3**

3.3.5 .Effect of rosuvastatin(high dose) on oxidative stress (MPO)

expression scores of MPO was more significant decreased with high dose rosuvastatin treatment in colitis group as shown in **table 3.3**

Table 3-3. Comparison of Clinical, microscopic score, and oxidative stress parameters in Acetic acid induce colitis group and high dose rosuvastatin group .

Variable	Acetic acid induce group	High dose rosuvastatin group	*P value*
DIA	9.89 ± 0.5	3.00 ± 0.6	<0.01**
W/L	0.21 ± 0.02	0.13 ± 0.01	<0.01**
HISTO Score	9.60 ± 2.9	3.00 ± 0.9	<0.05*
MACRO Score	3.20 ± 0.4	1.40 ± 0.5	<0.05*
IL-6	79.15 ± 7	37 ± 2.4	<0.01**
TNF-α	790 ± 73	470 ± 42	<0.01**
MPO	100 ± 14	50 ± 4	<0.01**
CRP	8.05 ± 0.3	3.4 ± 0.7	<0.01**

3.4. Effect of atorvastatin (low dose) on acetic acid –induced colitis in mice

3.4.1. clinical parameters

Low dose atorvastatin treatment exhibited a significant decreased in colon weight/ colon length ratio as compared with in colitis group as shown in **table3.4**

Disease activity index (DAI) of colonic injury was significant decreased with low dose atorvastatin treatment compared with in colitis group as shown in **table 3.4**

Low dose atorvastatin also significant decreases macroscopic score of colonic mucosal injury as compared with in colitis group as shown in **table 3.4**

3.4.2. Effect of atorvastatin (low dose) on cytokines (TNF-α and IL6). Low dose rosuvastatin treatment was significant decrease expression scores of(IL-6 and TNF-a) as compared to in colitis group as shown in **table 3.4**

3.4.3 Effect of low dose atorvastatin on microscopical score Histopathological score reflective of colonic injury was significant decreased with low dose atorvastatin treatment compared with in colitis group as shown in **table 3.4**

3.4.4. Effect of low dose atorvastatin on C-reactve protin (CRP) CRP was significant decreased in atorvastatin(low dose) group as compared with in colitis group as show in **table 3.4**

3.4.2. Effect of atorvastatin (low dose) on cytokines (TNF-α and IL-6). Low dose rosuvastatin treatment was significant decrease expression scores of(IL-6 and TNF-α) as compared to in colitis group as shown in **table 3.4**
3.4.5. Effect of atorvastatin (low dose) on oxidative stress (MPO) expression scores of MPO was significant decreased with low dose treatment atorvastatin as compared with in colitis group as shown in **table 3.4**

Table 3-4. Comparison of Clinical, microscopic score, and oxidative stress parametersin Acetic acid induce colitis group and low dose atorvastatin group

variable	Acetic acid induce group	Low dose atorvastatin group	*P value*
DIA	9.89 ± 0.5	3.90 ± 0.7	<0.01 **
W/L	0.21 ± 0.02	0.14 ± 0.02	<0.01**
HISTO Score	9.60 ± 2.9	6.00 ± 1.2	<0.01**
MACRO Score	3.20 ± 0.4	1.80 ± 0.4	<0.05 *
IL-6	79.15 ± 7	46 ± 5	<0.01**
TNF-α	790 ± 73	594 ± 78	<0.05 *
MPO	100 ± 14	78 ± 7	<0.05 *
CRP	8.05 ± 0.3	4.3 ± 0.4	<0.01**

3.5. Effect of atorvastatin (high dose) on acetic acid –induced colitis in mice

3.5.1. clinical parameters

High dose atorvastatin treatment was more significant than low dose decreased in colon weight/ colon length ratio as compared with in colitis group as shown in **table 3-5**

Disease activity index (DAI) of colonic injury was highly significant decreased with high dose atorvastatin treatment as compared with in colitis group as shown in **table 3-5**

High dose atorvastatin also more significant than low dose in decrease macroscopic score of colonic mucosal injury as compared with in colitis group as shown in **table 3-5**

3.5.2. Effect of low dose atorvastatin on microscopical score

Histopathological score reflective of colonic injury was more significant decreased with high dose atorvastatin treatment than low dose compared with in colitis group as shown in **table 3-5**

3.5.3 Effect of high dose atorvastatin on C-reactve protin (CRP)

CRP was highly significant decreased in atorvastatin(high dose) group as compared with in colitis group as show in **table 3-5**

3.5.4 .Effect of atorvastatin (high dose) on cytokines (TNF-α and IL6).

High dose atorvastatin treatment was more significant decrease expression scores of(IL-6 and TNF-a)than low dose as compared with in colitis group as shown in **table 3-5**

3.5.5 .Effect of atorvastatin (high dose) on oxidative stress (MPO)

expression scores of MPO was more significant decreased with high dose atorvastatin treatment as compared with in colitis group as shown in table 3-5.

Table 3-5. Comparison of Clinical, microscopic score, and oxidative stress parameters in Acetic acid induce colitis group and high dose atorvastatin group

variable	Acetic acid induce colitis group	High dose atorvastatin group	*P value*
DIA	9.89 ± 0.5	2.1 ± 0.4	<0.01**
W/L	0.21 ± 0.02	0.12 ± 0.01	<0.01**
HISTO Score	9.60 ± 2.9	2 ± 0.9	<0.01**
MACRO Score	3.2 ± 0.4	1.2 ± 0.4	<0.01**
IL-6	79.15 ± 7	32 ± 2	<0.01**
TNF-α	790 ± 73	420 ± 47	<0.01**
MPO	100 ± 14	45 ± 5	<0.01**
CRP	8.05 ± 0.3	2.5 ± 0.3	<0.01**

3.6. Effect of prednisolone on acetic acid –induced colitis in mice

3.6.1. Gross morphological changes

Treatment with standard drug prednisolone caused highly significant decreased in colon weight/ colon length ratio as compared with in colitis group .

Furthermore DAI of colonic injury was highly significant decreased with prednisolone treatment versus in colitis group.

prednisolone also caused highly significant decreases in macroscopic score of colonic mucosal injury as compared with in colitis group.

3.6.2. Effect of prednisolone on microscopical score

Histopathological score was significantly decreased with prednisolone treatment as compared with in colitis group.

3.6.3 Effect of prednisolone on C-reactive protein (CRP)

CRP was significant decreased in prednisolone group compared with in colitis group.

3.6.4 .Effect of prednisolone on expression score of cytokines (TNF-α and IL-6)

Moreover treatment with prednisolone significantly decreased the expression scores of TNF-αas compared to colitis group.

furthermore treatment with prednisolone significantly decreased the expression scores of IL-6 as compared in colitis group.

3.6.5 .Effect of prednisolone on expression score of oxidative stress (MPO)

The expression scores of MPO was highly significant decreased with prednisolone treatment versus in colitis group.

Table 3-5. Comparison of Clinical, microscopic score, and oxidative stress parameters in Acetic acid induce colitis group and prednisolone group

variable	Acetic acid induce colitis group	Prednisolone group	*P value*
DIA	9.89 ± 0.5	2.7 ± 0.0	<0.01**
W/L	0.21 ± 0.02	12 ± 0.02	<0.01**
HISTO Score	9.60 ± 2.9	1.5 ± 0.0	<0.01**
MACRO Score	3.2 ± 0.4	0.5 ± 0.0	<0.01**
IL-6	79.15 ± 7	30 ± 4.9	<0.01**
TNF-α	790 ± 73	365 ± 63	<0.01**
MPO	100 ± 14	36 ± 4	<0.01**
CRP	8.05 ± 0.3	2.3 ± 0.5	<0.01**

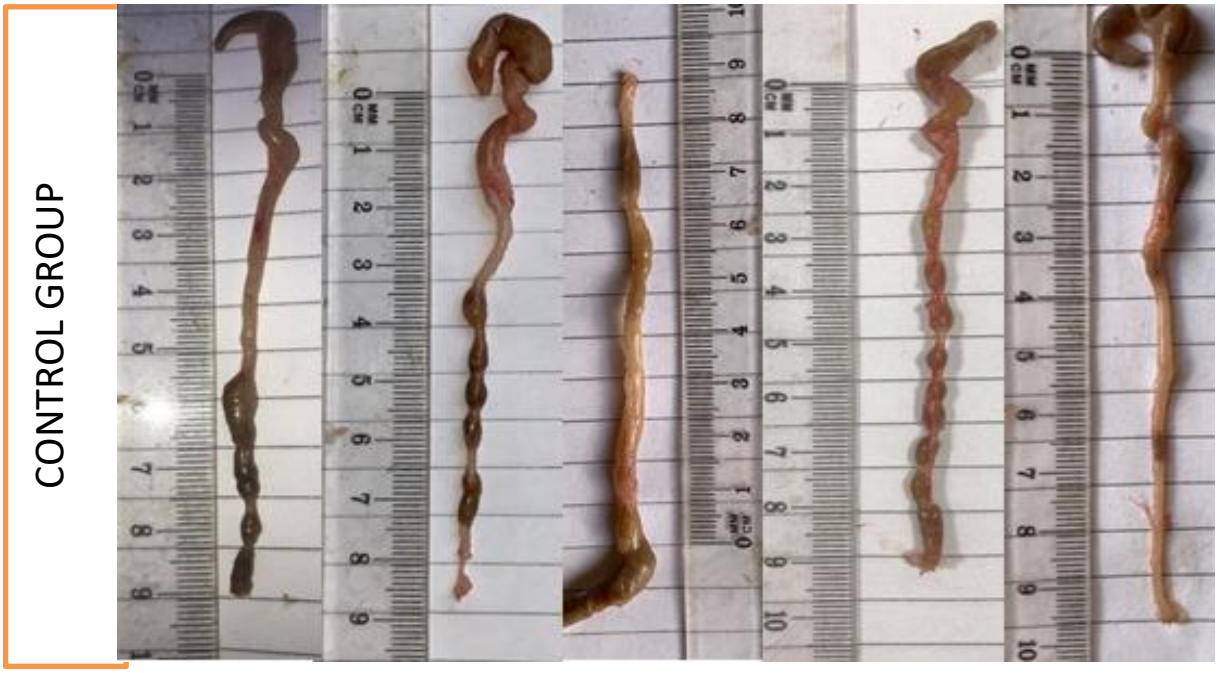
Table 3.6. Comparision of histo pathlogical score in control and study groups. N=10

Scors	Cont	Ind	Pred	L.D Ros	H.D Ros	L.D Ator	H.D Ator
Destraction of epithelium and/orglandular crypt	0	7	0	2	6	4	1
Dilation of orglandular crypt	0	6	0	2	1	0	0
Depletion and loss of globlet cell	0	5	1	2	1	1	0
Inflammatory cell infiltration	0	L 9 N 7	L 1 N 0	L 6 N 4	L4 N 7	L 4 N 5	L 3 N 3
Edema	0	6	1	3	1	3	1
Hemorrhagic mucosa	0	6	0	1	0	0	0
Crypt abscess	0	5	0	3	1	0	0
Apoptosis	0	0	0	0	0	0	0
Dysplasia	0	5	0	0	1	3	1

*L :Lymphocyte

* N:Neutrophil

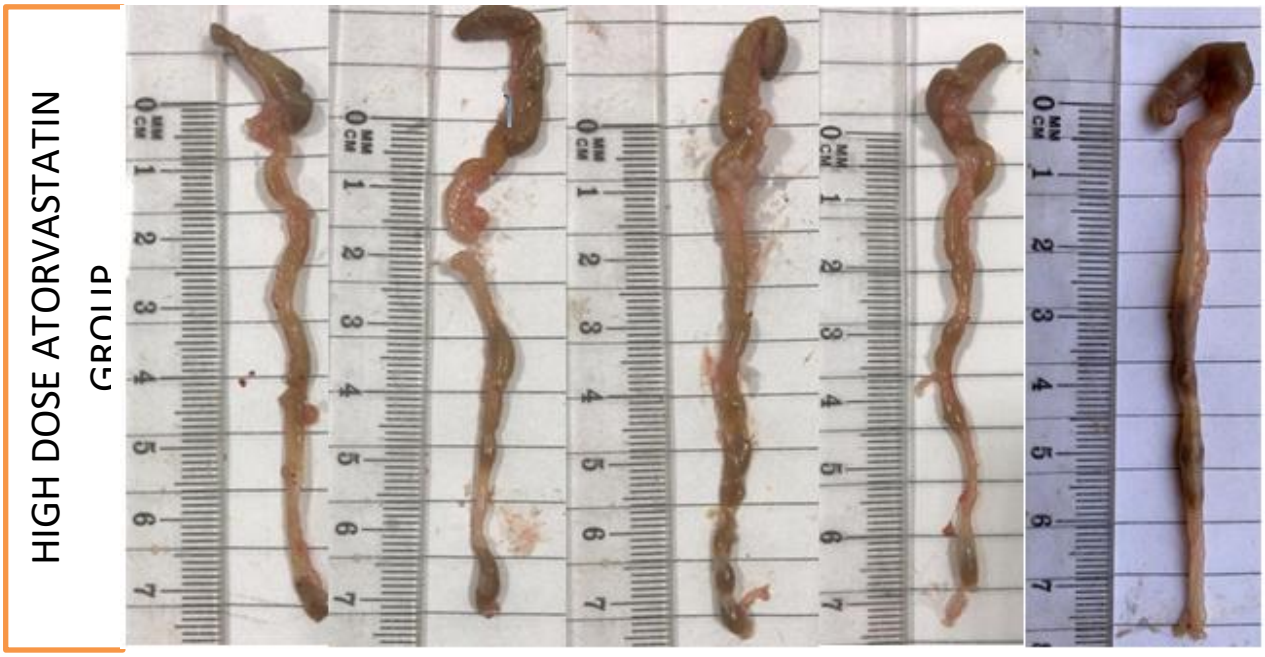
Figure(3.3) :show length of mice colon in Control group



Figure(3.4) :show length of mice colon in Prednisolon group

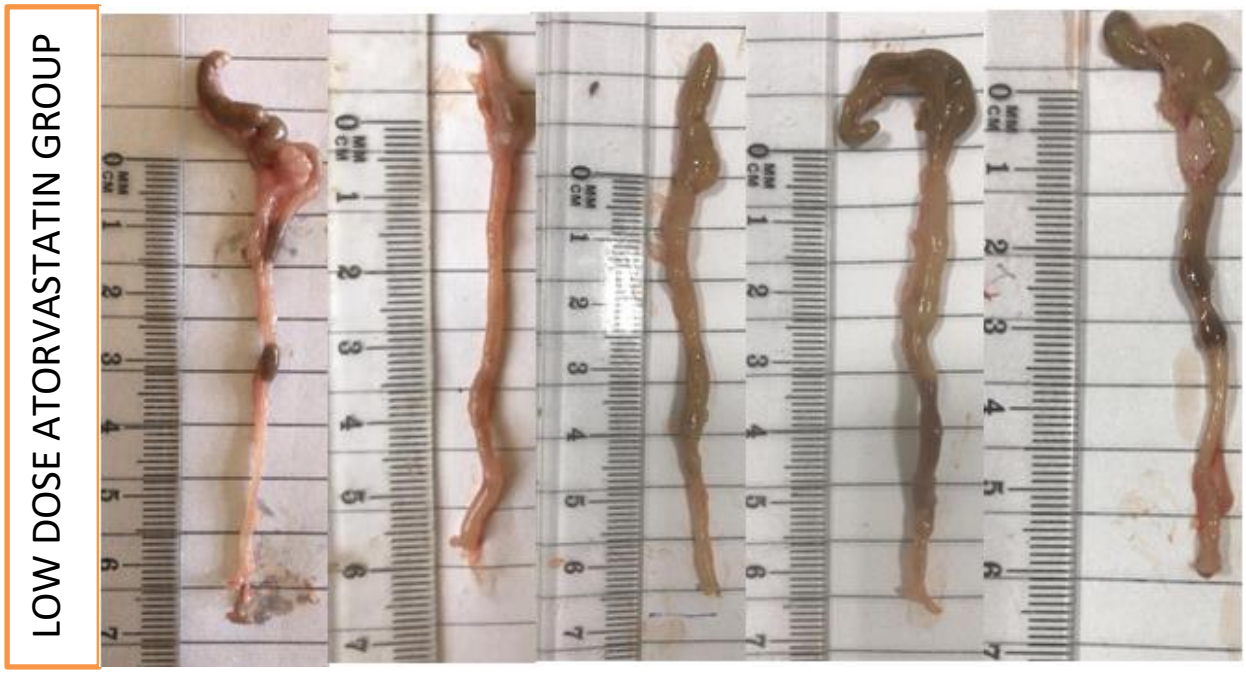
Figure(3.5) :show length of mice colon in Induction group

Figure(3.6) :show length of mice colon in High Dose Atorvastatin group

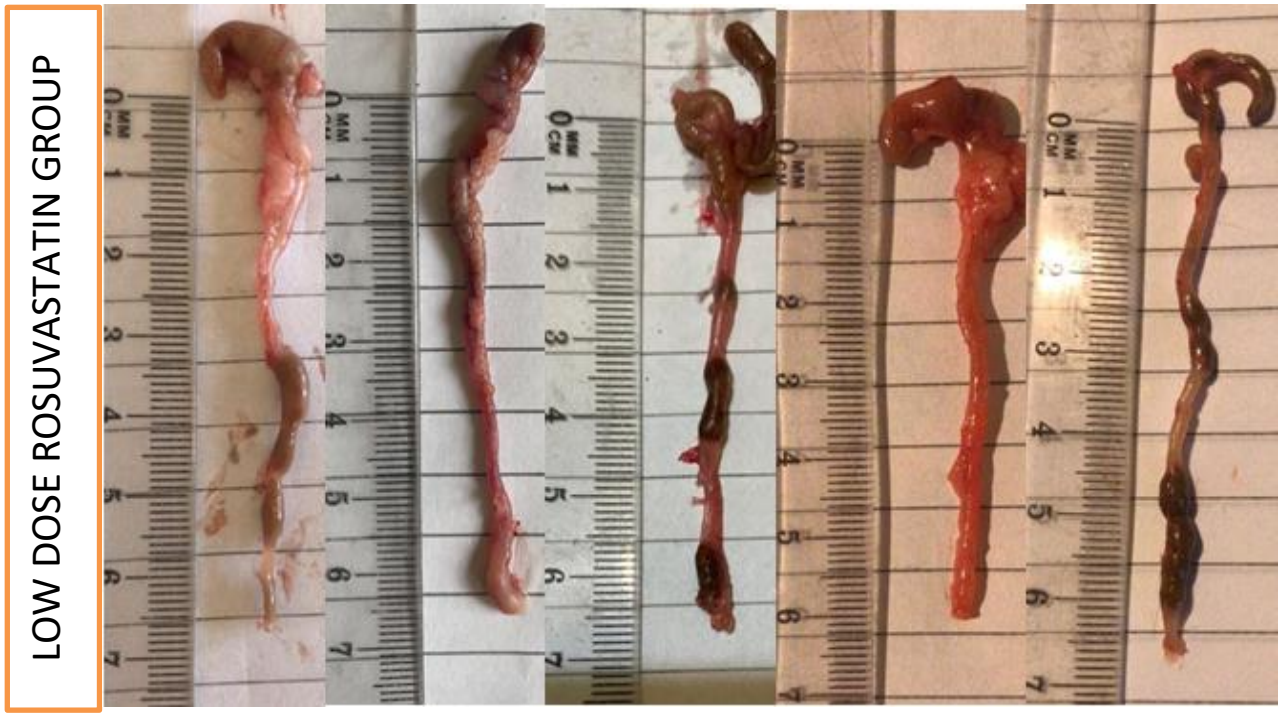


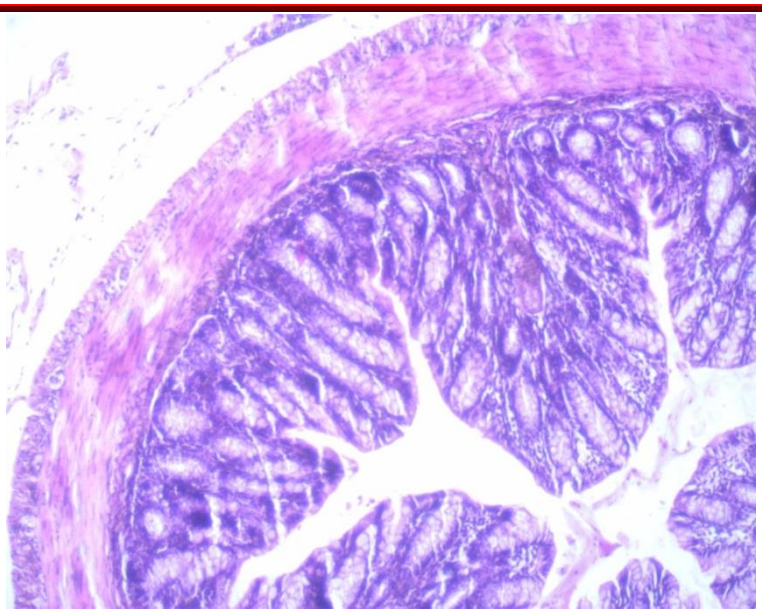
Figure(3.7) :show length of mice colon in High Dose Rosuvastatin group

Figure(3.8) :show length of mice colon in High Dose Rosuvastatin group



Figure(3.9) :show length of mice colon in Low Dose Rosuvastatin group





Figure(3.10):Control group sample 10 Normal Histology X 10 H and E

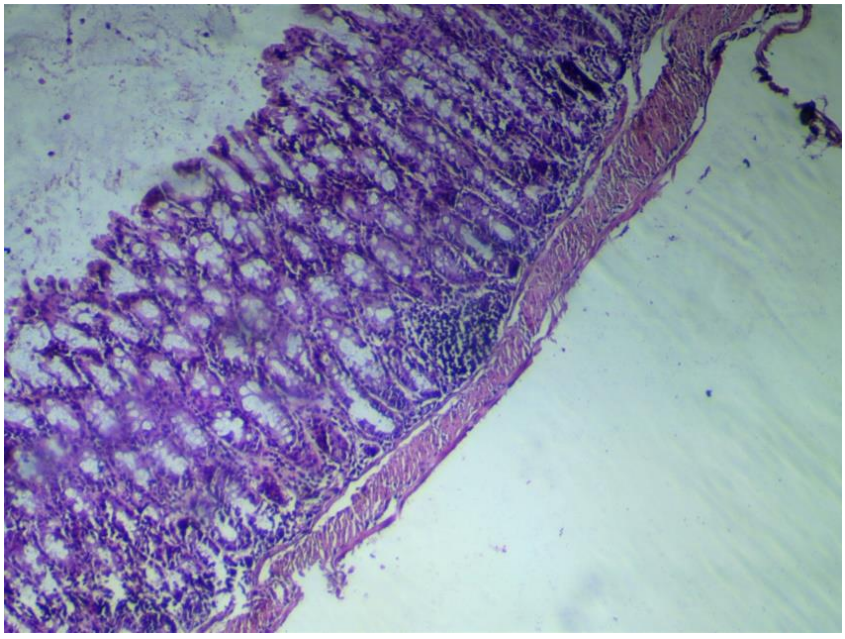
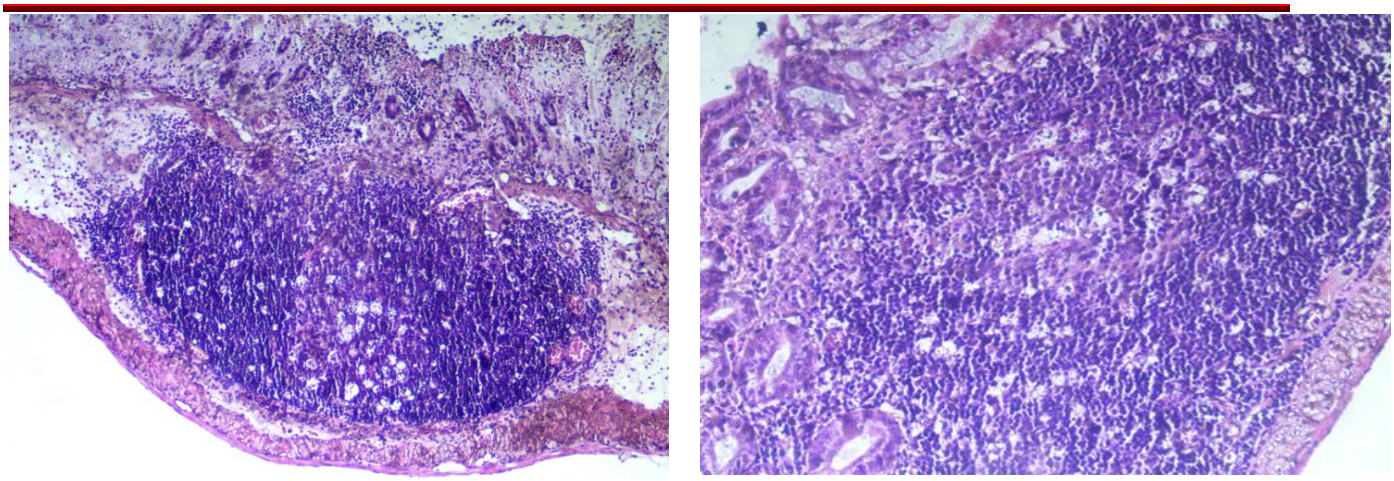


Figure (3.11): Control group sample 7 Normal Histology with Mucosal Primary Lymphoid Follicle X 10 H and E



A B

Figure(**3.12**):Induction groupsample3 histological section through colonic wall showing Tangible Body Germinal Centre Secondary Lymphoid Follicle;A: 10X ;B:20X; H and E

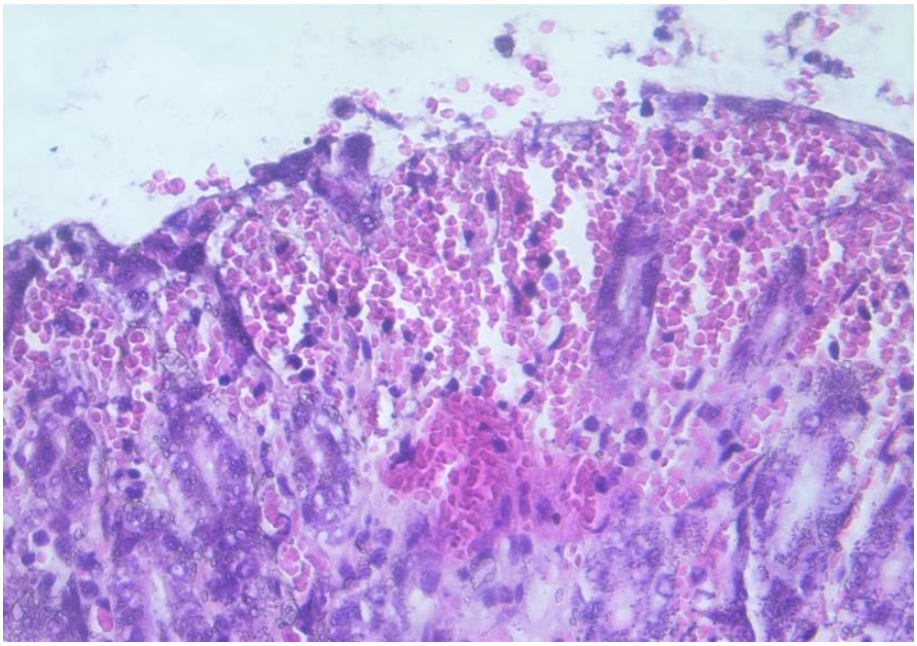


Figure (3.13): Induction group sample 3 Histological section through colonic wall showing Hemorrhage and Slughing ; 40X ; H and E

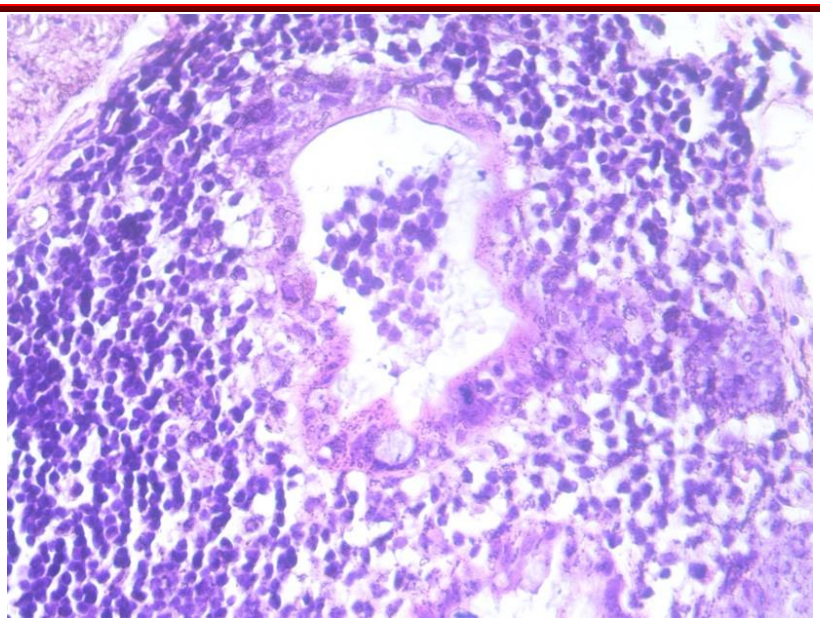
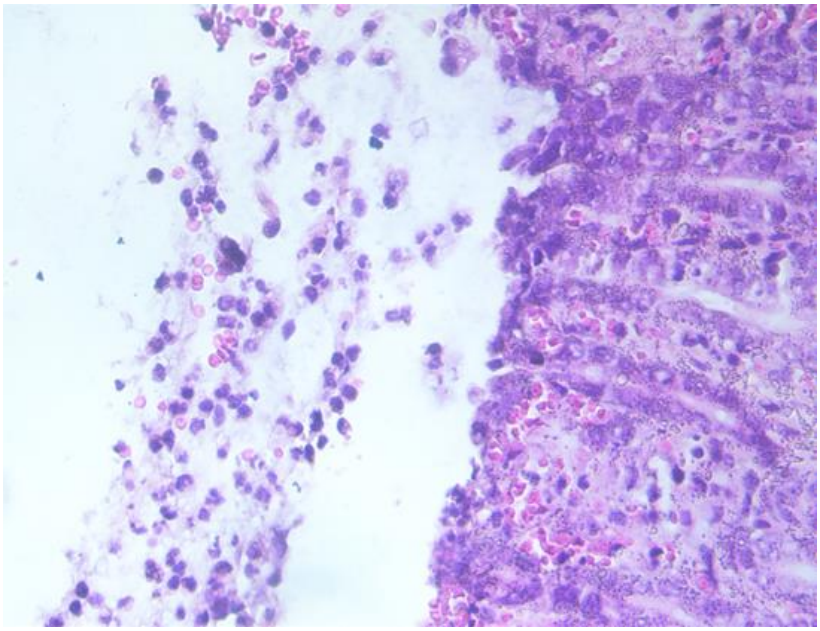


Figure (3.14): Induction group sample 2 Histological section through colonic wall showing Crypt Abcess; 40X ; H and E



Figure(3.15): induction group sample 3 Histological section through colonic wall showing Luminal Neutrophils and Lymphocyte infltration IN; 40X ; H and E

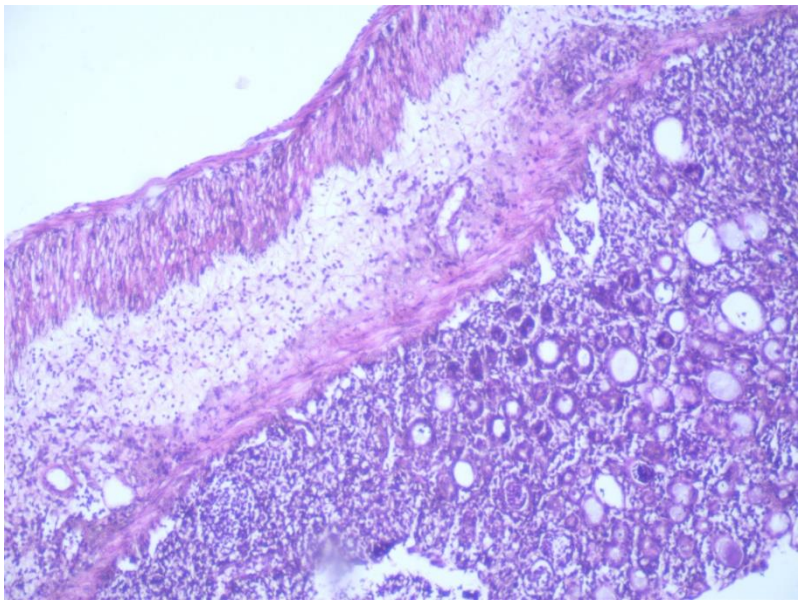


Figure (3.16): Low dose Rosuvastatin group sample 14 Few Inflammetory Cell and Submucosal Odema x 10 H and E.

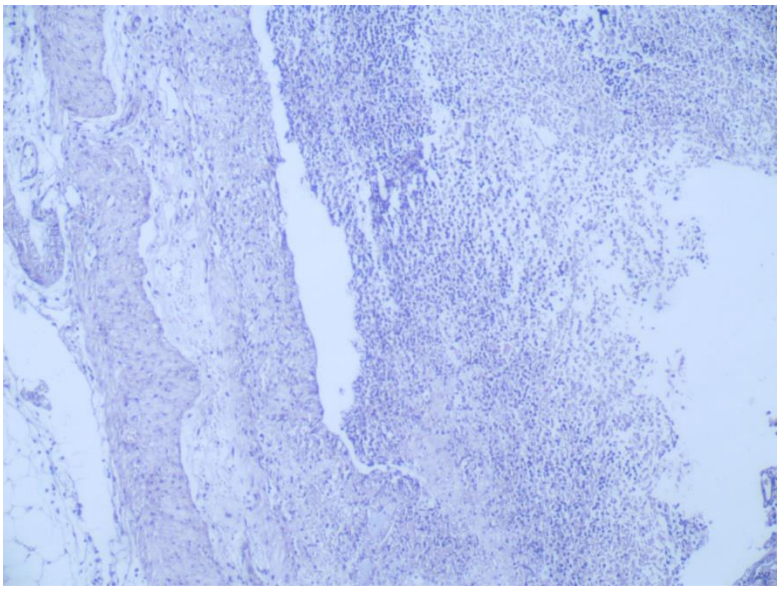


Figure (3.17): High dose Rosuvastatin group sample 2 Destrution of epithelial and Neutrophils Infltration x 10 H and E.

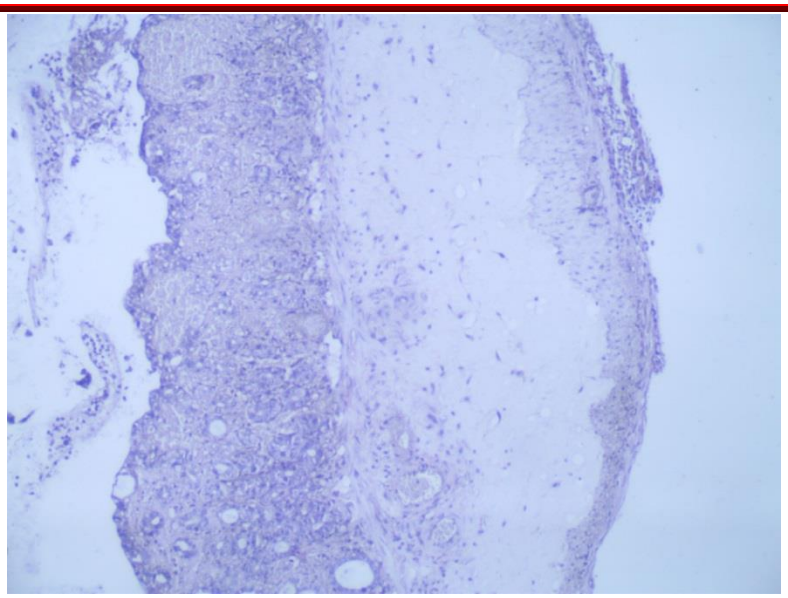


Figure (3.18):Low dose Atorvastatin sample 2 Few Inflammetory Cell and Submucosal Odema x 10 H nd E.

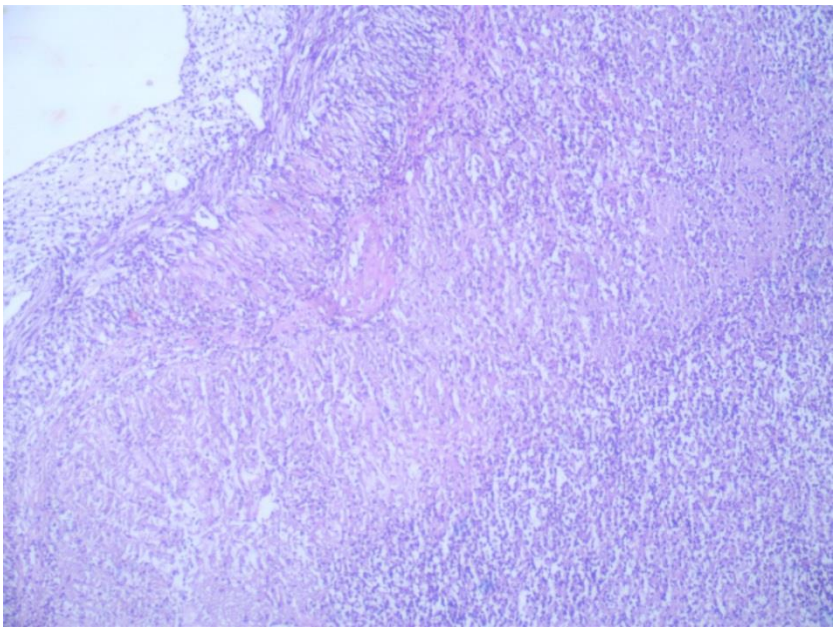
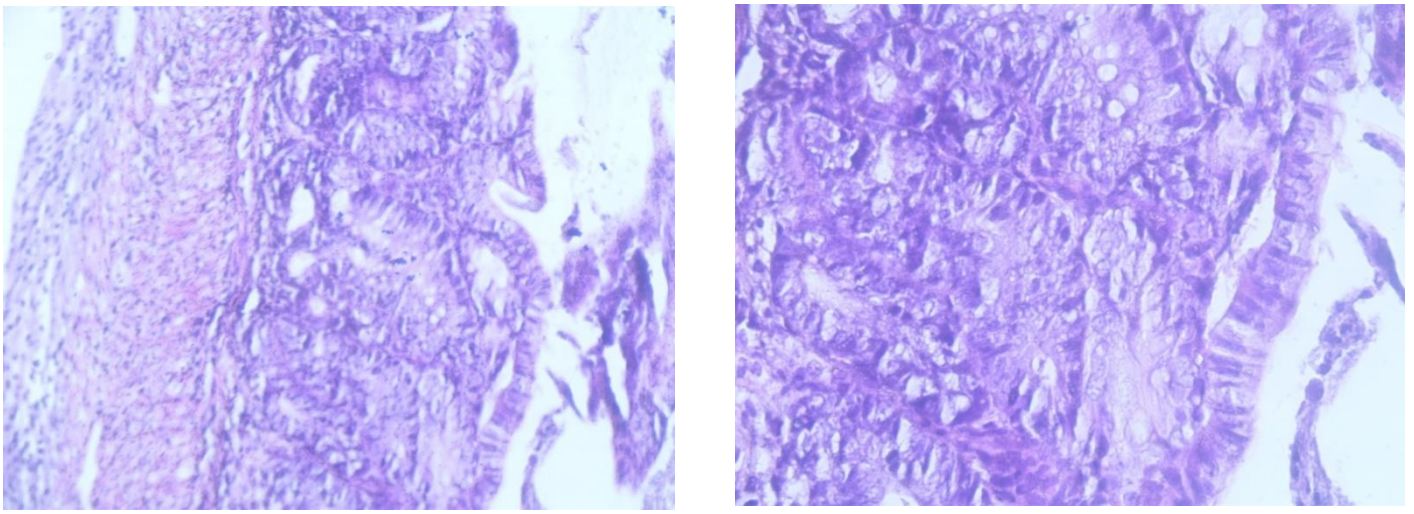


Figure (3.19): Low dose Atorvastine sample 5 Inflammetory Cell Infltration of all intestine wall layesrs x 10 Hand E



A B

Figur.(3.20): High dose Atorvastatin sample 5Surface Epithelial Dysplasia; A:20X ;B: 40X; HandE

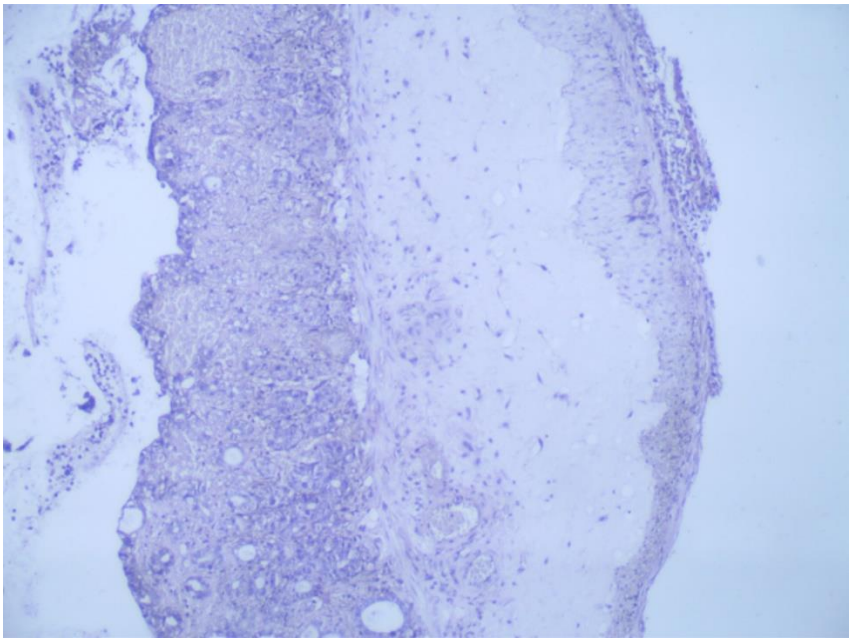
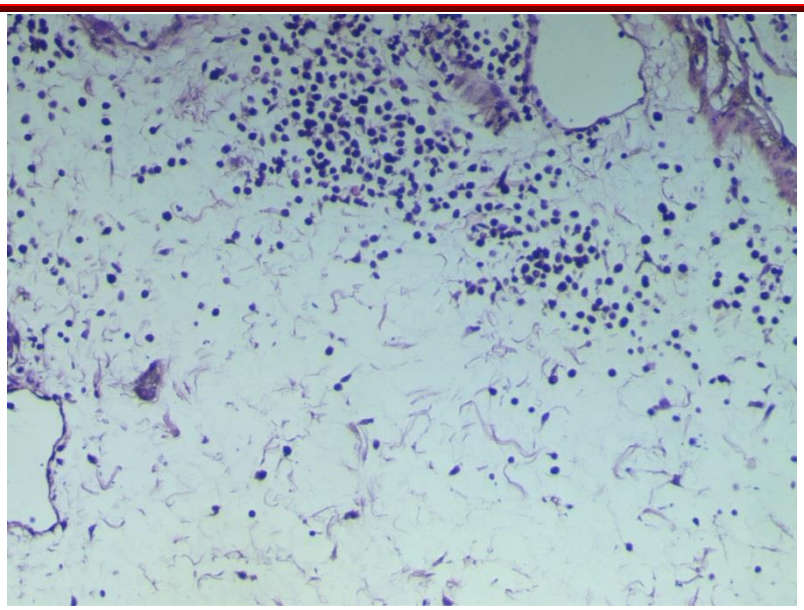
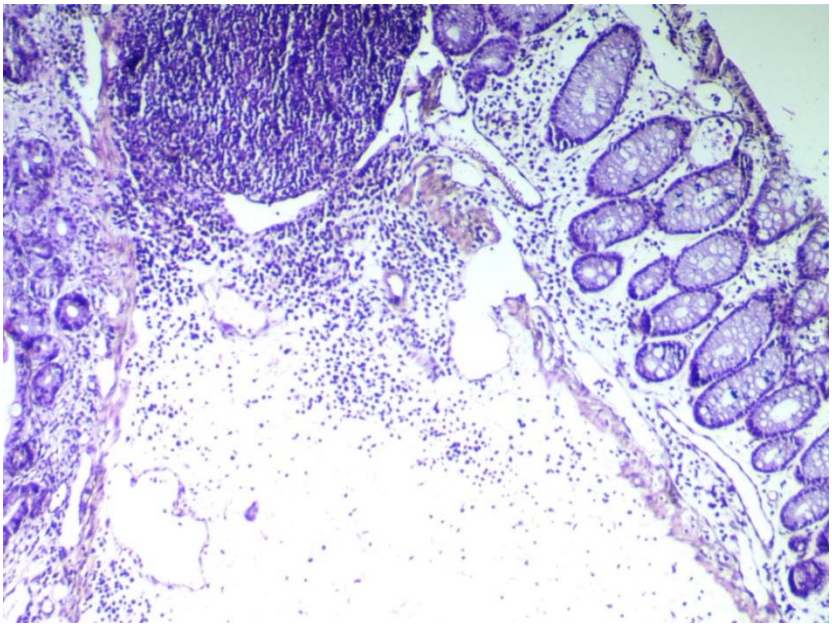


Figure (3.21): Low dose Atorvastatin group sample 2 Few Inflammetory Cell and Submucosal Odema; x10 HandD



Figure(3.22): Prednisolone group sample8 Histological section through colonic wall showing Submucosal Odema and Lymphocyte; 20X ; H and E



Figure(3.23): Prednisolone group sample8 Histological section through colonic wall showing Mucosal Lymphoid Follicle Submucosal Odema and Lymphocyte ; 10X ; H and E

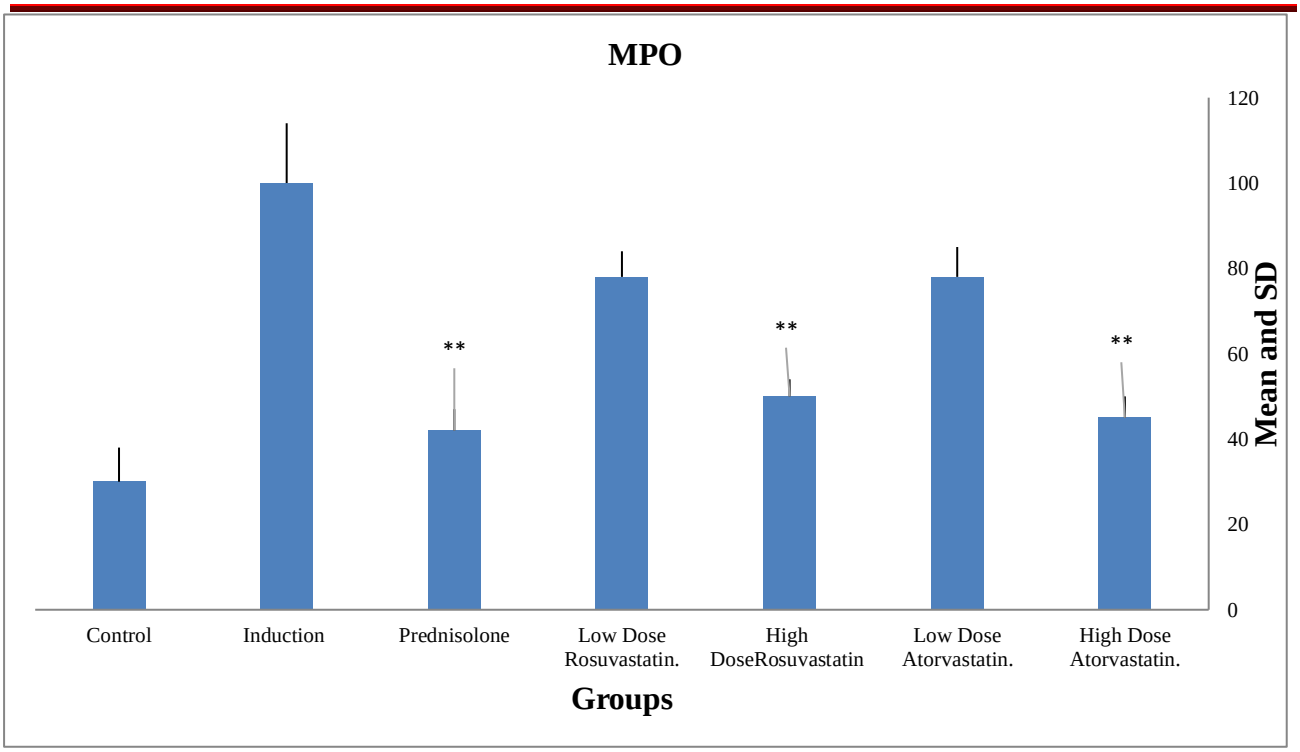


Figure 3-24: myeloxyperoxide (MPO) concentration in control and study Groups. Comparison between means of MPO in all groups. . (N=10), **=p<0.01

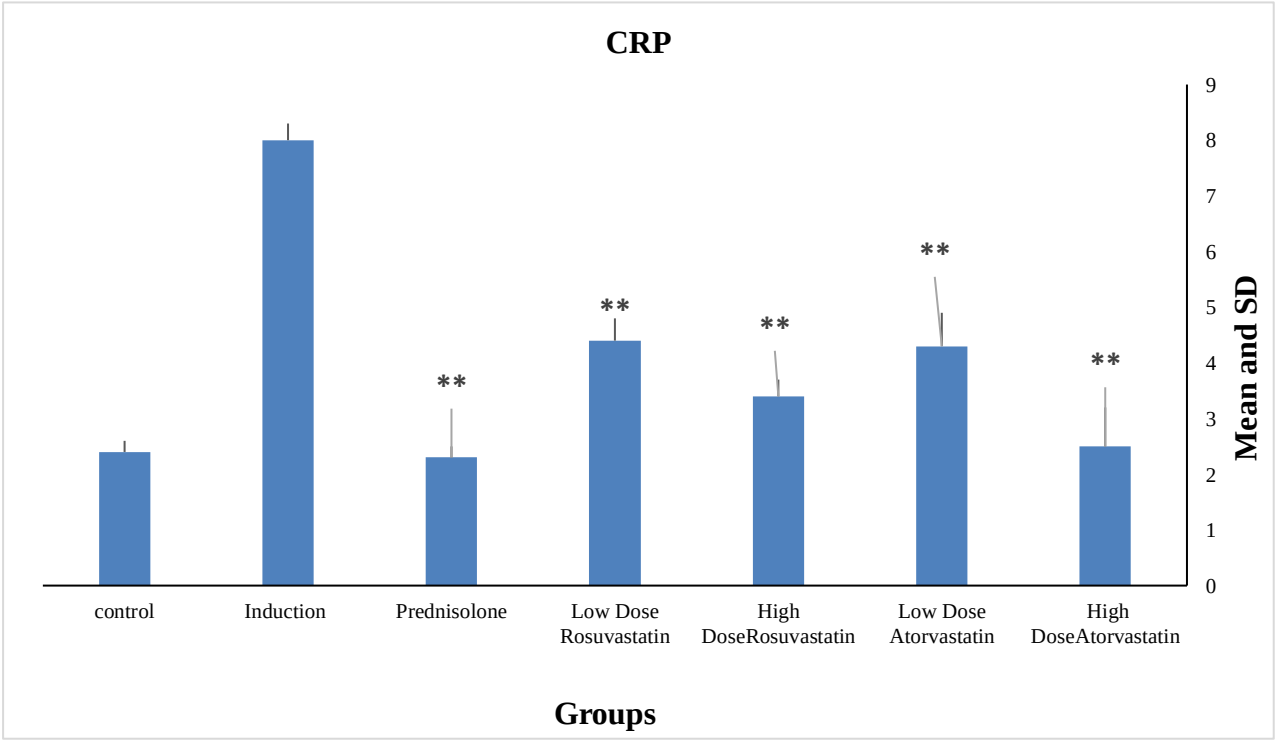


Figure 3-25: C reative protein(CRP) concentration in control and all study Groups. Comparison between means of CRP in all groups. . (N=10), **=p<0.01

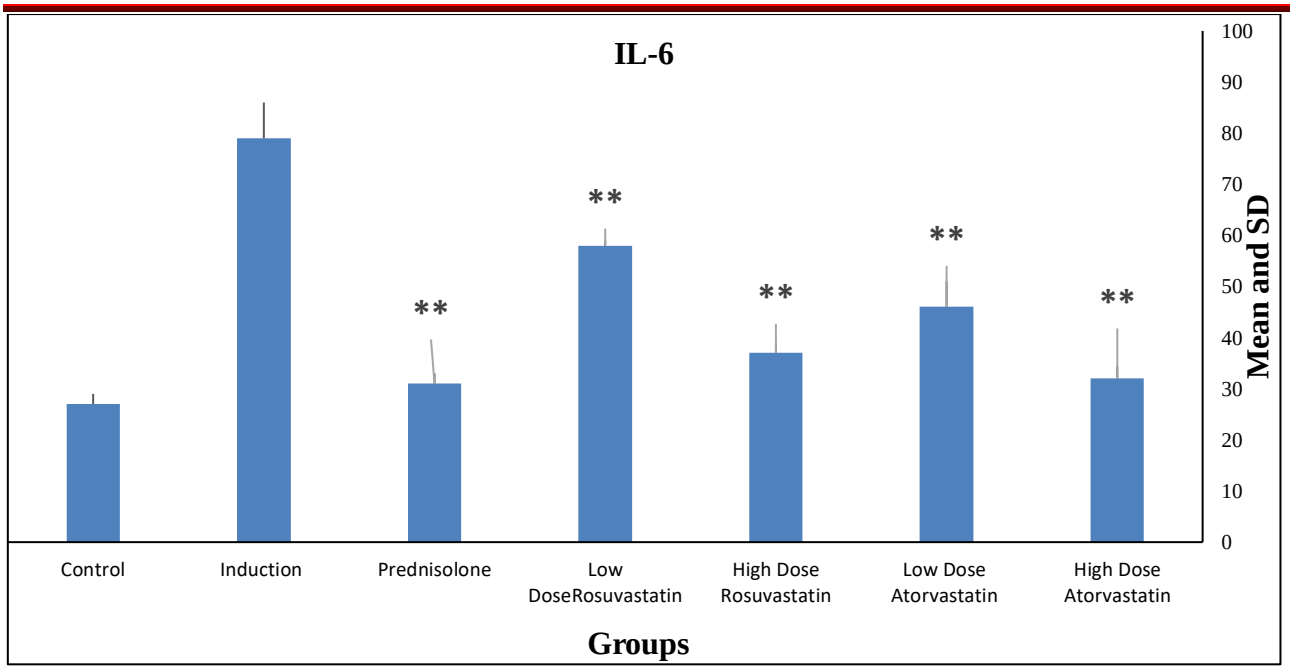


Figure 3-26: Interleukins- 6 (IL-6)score in control and study Groups. Comparison between means of IL-6 in all groups. . (N=10), **=p<0.01

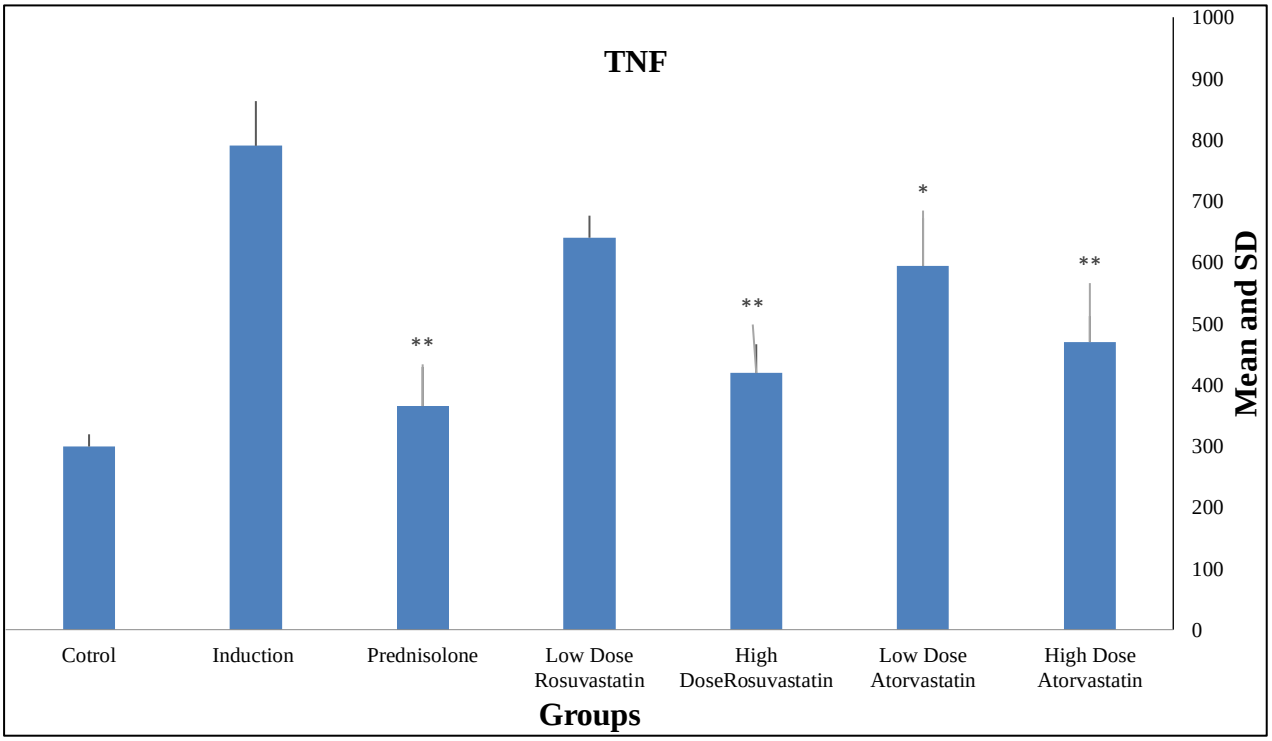


Figure 3-27: Tumor necrosis factor alpha (TNF-α)score in control and study Groups. Comparison between means of IL-6 in all groups. . (N=10), **=p<0.01, *=P<0.05

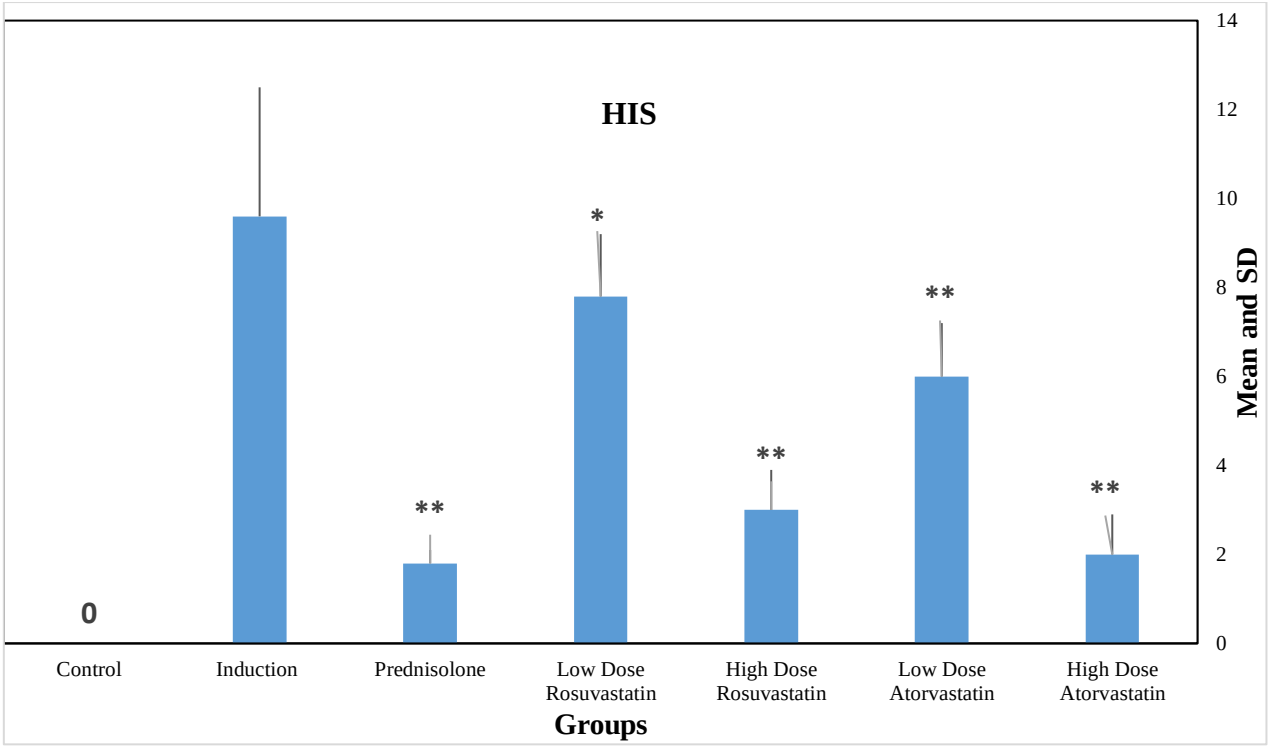


Figure 3-28:Histopath (HIS)score in contro and all study Groups. Comparison between means of HIS in all groups. . (N=10), **=p<0.01, *=p<0.05

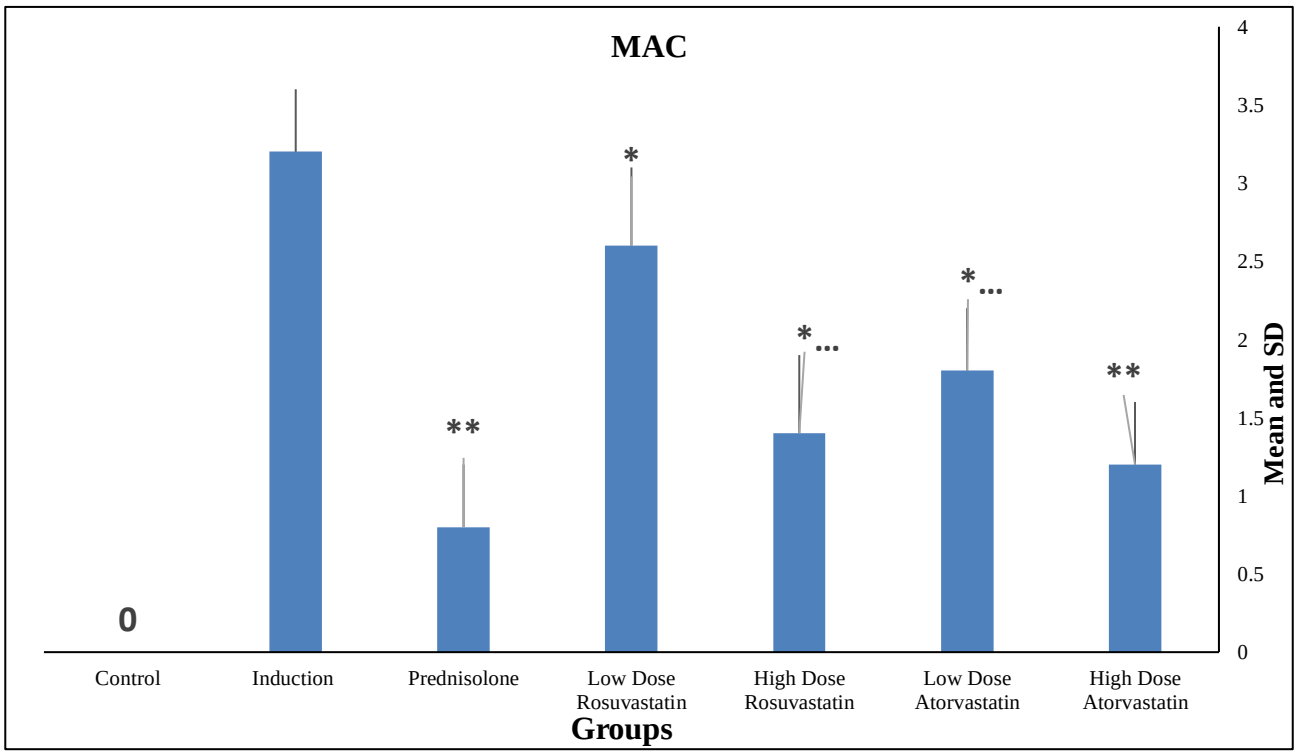


Figure3-29 :Macroscopic (MAC) score in control and all study Groups. Comparison between means of MAC in all groups. . (N=10), **=p<0.01, *=p<0.05

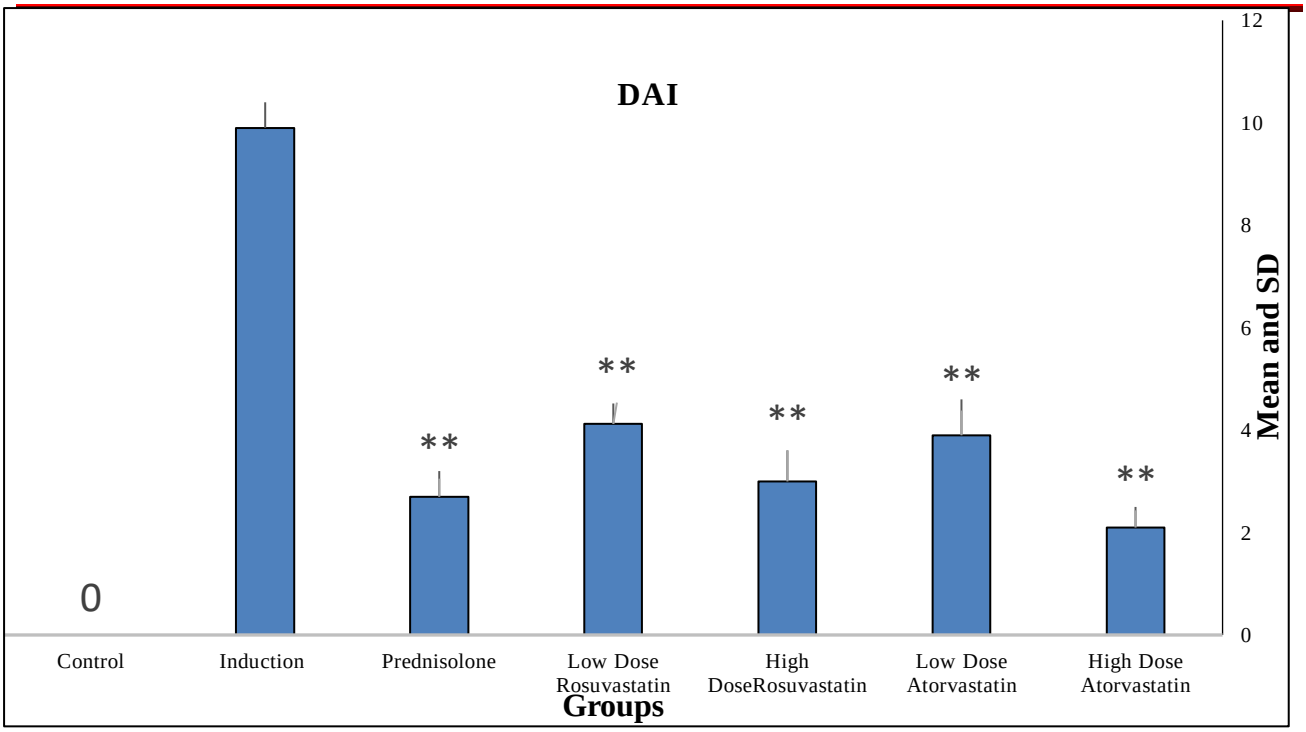


Figure 3-30 : Deasese activity index (DAI) score in control and study Groups Comparison between means of DAI in all groups. . (N=10), **=p<0.01

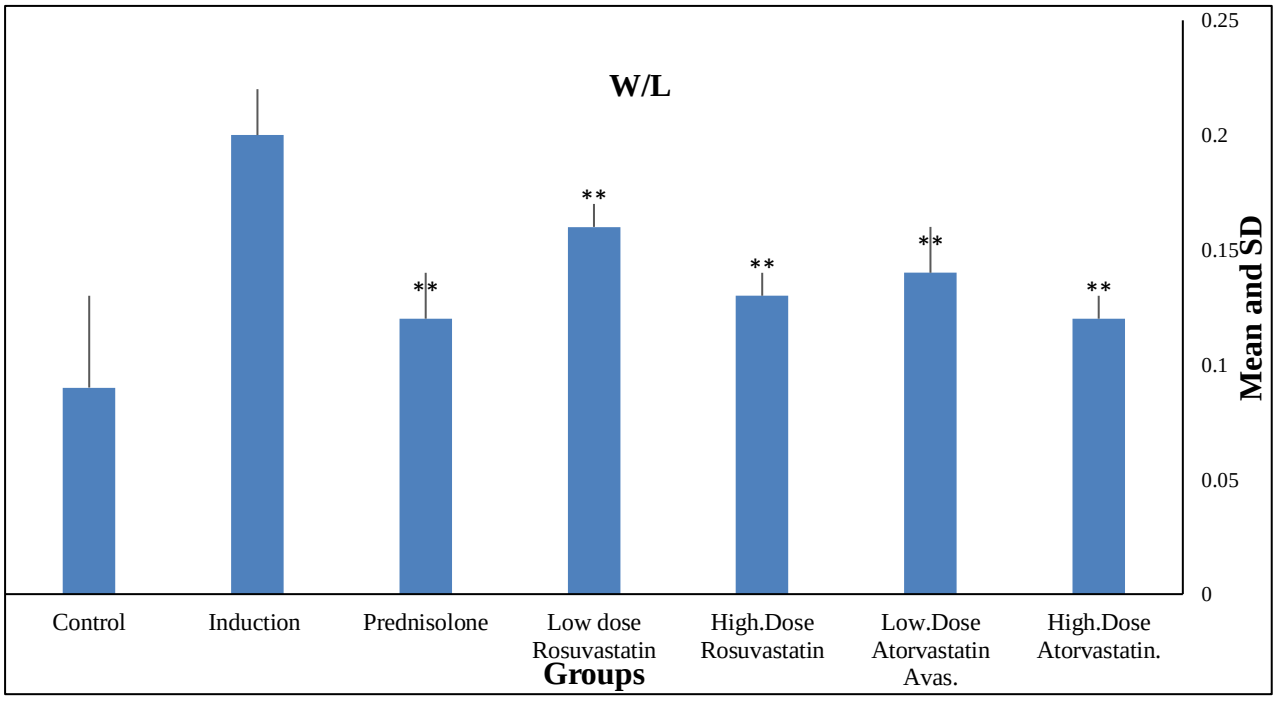


Figure 3-31: Weight/Length (W/L) score in control and study Groups Comparison between means of W/L in all groups. . (N=10), **=p<0.01

4. Discussion

Ulcerative colitis is a disorder of the intestines characterized by chronic refractory inflammation and ulceration of the colon, and usually, with a relapsing form, several drugs have been developed for UC with no effective line of treatment, due to the inadequate responses and significant adverse effects of immunosuppressant. Therefore, novel and safer therapies with more curative efficacy are needed (Ungaro *et al.*, 2017).

4.1. Effect of acetic acid on colonic mucosa in mice

A major advance in the understanding of the aetiology of UC has arisen from experimental studies. In this respect, The acetic acid model of ulcerative colitis through enemas exhibits advantages over other experimental models, include low cost, low mortality with easily reproducible lesions and mimics to the pattern of inflammation in human UC, especially in terms of pathogenesis, histopathological features and inflammatory mediator profile (Randhawa *et al.*, 2014). The mechanism by which acetic acid induces colitis mediated through the entrance of protonated form of acid into the epithelium then separates to release proton which causes acidification of intracellular space that may cause intensive colitis (Vinod Prabhu and Guruvayoorappan, 2014).

Major causative factors in the initiation of human UC such as excessive oxidative stress, enhanced microvascular permeability, sustained immunocytes infiltration and increased inflammatory mediators output were all involved in the induction of this animal model, therefore it is suitable for the investigation of ulcerative colitis pathogenesis and evaluation of the novel therapeutic options of this disease (Hagar *et al.*, 2007).

Acetic acid caused an increase in mean **DAI** in the untreated colitis rats. our findings were in agreement with other previous results (Amirshahrokhi, Bohlooli and Chinifroush, 2011);(Owusu *et al.*, 2020), elevation **DAI** scores in the colitis group

evidenced by reduce body weight, decrease in food intake, and increase in diarrhoea with or without blood.

In our study acetic acid causes an increase in mean segment weight of colon and colon **weight/ length ratio** which is consistent with the finding of (Kumar *et al.*, 2014) ;(Mehesen, 2015). The weight of the colon is raised due to the infiltration of the inflammatory cells, goblet cell hyperplasia, oedema and necrosis(Kondamudi *et al.*, 2014)(Moura *et al.*, 2015). Colon length shrinkage score was considered as an indicator of inflammation and functional changes (Nabarawi, 2014).

The present work demonstrated characteristic **histological** features in untreated colitis, essentially loss of intestinal crypt architecture and sloughing of intestinal cells, reduced goblet cell number and presence of different inflammatory cell infiltration. These results concord with recent findings of (Lean *et al.*, 2015); (El-Galil and ElGhamrawy, 2015).

Moreover, acetic acid increases the **macroscopic** score of colonic mucosa which is demonstrated as erythema, oedema, erosion and tissue ulcerations and/or necrosis nearly similar to observations of (Amirshahrokhi, Bohlooli and Chinifroush, 2011) ;(Owusu*etal.*,2020).

Our study who described the increased level of expression of pro-inflammatory cytokines (**TNF-α, and IL-6)** and these data support the concept of the efficacy of this model as a means of reproducing observations made on ulcerative colitis in humans, where the increase of these pro-inflammatory cytokines is related to the severity of disease (Bertevello *et al.*, 2005). and their presence had been previously correlated with colonic inflammation in UC. **TNF-α** induces a variety of inflammatory genes and promotes the expression of pro-inflammatory cytokines, it is a crucial pro-inflammatory cytokine liberated from the lymphocytes and macrophages in the initial step of inflammation, also, it can produce different mediators which responsible for severe inflammation that contributes to the

development of UC**, IL-6** plays a pivotal role in the pathogenesis of UC by stimulating neutrophil infiltration and chemotaxis to produce tissue necrosis and destruction. All that showed that level of **TNF-α and IL-6** was significantly increased in UC(Gupta, Motiwala and Mahajan, 2018);(Xiao *et al.*, 2016).

The present study demonstrated that the colitis group was associated with"a significant increase in the level of **MPO** and levels in the colonic tissue. This finding is comparable with an observation of (Olamilosoye *et al.*, 2018);(Arafa *et al.*, 2020) (Tanideh *et al.*, 2020); (Cagin *et al.*, 2016) has indicated that oxidative stress results from the shift of equilibrium between the pro-oxidant and anti-oxidant systems in favour of the pro-oxidant system which result of excessive production of free oxygen radicals and neutrophil infiltration". Myeloperoxidase is a hemoprotein enzyme abundantly released from the neutrophils' granules by inflammatory stimuli that catalyze the formation of several reactive species (Nussbaum *et al.*, 2013). The CL^{-} is produced by the **MPO** enzyme, which leads to the production of hypochlorous acid (HOCL). Both **MPO** and HOCI cause lipid peroxidation and tissue damage. Malondialdehyde" is the toxic end-product of lipid peroxidation"(Cagin *et al.*, 2016).

4.2 Effect of atorvastatin(low dose) on acetic acid-induced colitis in mice

Administration of atorvastatin caused a significantly reduce the body weight and decrease in AA-induced ulcer area, UI with improvement in ulceration formation and clinical symptoms of UC in the form of diarrhoea and rectal bleeding which were reflected in declining of **DAI** in a dose-dependant manner (Soliman *et al.*, 2019) (Kanagarajan *et al.*, 2008).

Atorvastatin in low dose decreasing **weight/length** score in colitis mice by reducing the colon shortening associated with the induced colitis (Park *et al.*, 2004). and therapeutic intraperitoneal administration of atorvastatin suppressed most of the **pathological** manifestations of colitis.

In our experiments, overall **microscopic** and most **macroscopic** evaluations were significantly reduced in an atorvastatin treated group compared with the colitis group.And atorvastatin in this dose showed deacrese colonic damage induced by Acetic acid (Aktunc *et al.*, 2011)..the Histologic findings showed fewer inflammatory cells in the colons of atorvastatin treated mice,the diffuse inflammatory infiltrate involved mucosa and submucosa and submucosal edema, also entire crypts was lost (Elkatary *et al.,* 2015) .

Atorvastatin led to a significant lowering of **CRP**. Its significantly lowered compared to colitis group but still elevated than normal CRP is useful as a laboratory marker to predict prognosis and relapse in patients with IBD (Nabarawi, 2014).

Atorvastatin in dose 5 mg slightly reduces elevated pro-inflammatory cytokines**(TNF-a And IL6)** as compared in the induction group by inhibiting pro-inflammatory by blocking NF-κB signalling in intestinal epithelial cells on an experimental murine colitis model (Aktunc *et al.,* 2011).

The treatment with a low dose of atorvastatin decreased the expression of **MPO** (Rashidian *et al.*, 2016). the statin is effective in decreasing neutrophil infiltration and oxidant generation (i.e. superoxide and peroxynitrite) by the inflamed tissue. (Gedi, 2006).

4.3 Effect atorvastatin (high dose) in acetic acid-induce colitis in mice

Our results showed that administration of atorvastatin significantly reduced the severity of murine colitis as assessed by body weight loss, diarrhoea rectal bleeding all of that lead to reduced disease activity index **(DAI)** (Date, 2016).

Although statins increased **colonic length** (decreased colonic length can reflect colonic inflammation) in studies–(Bereswill *et al.,* 2010))(Cote-Daigneault *et al.,* 2015) (Kanagarajan *et al.,* 2008).

In high dose atorvastatin, the **microscopical** score significantly decrease than a low dose of this drug because the extent of inflammation involving mucosa only. with focal mucosal infiltrations of neutrophil lymphocytes and focal submucosal edema .while in low dose the inflammation and necrosis involves mucosa and sub mucosa, and the crypts were lost (Elkatary *et al.*, 2015).

Atorvastatin in high dose significantly reduces the **macroscopic** manifestation than low dose atorvastatin by decreasing severe mucosal injury, wall thickening, ulceration, oedema and necrosis. And atorvastatin dose in high dose group showed no epithelium damage (Elkatary *et al.*, 2015);(Rashidian *et al.*, 2016).

Atorvastatin reduced plasma levels of **C-reactive protein** (Grip and Janciauskiene, 2009) .**(CRP and IL-6)**significantly reduce in high dose than in low dose of atorvastatin (Niafar *et al.*, 2019).

High dose of atorvastatin was more reduced **TNF-α** than low dose group. It significantly inhibited the AA-induced increase in **TNF-α** expression in colonic tissue, which is commonly overexpressed in this model of colitis decreased the severity of the intestinal inflammation in the experimental colitis and caused a marked reduction of inflammation in the colon of patients with IBD(Rashidian *et al.*, 2016).

Atorvastatin treatment significantly reduced IL-17 level as we also observed on **TNF-α and IL-6** (crucial cytokine IL-17 is a pro-inflammatory cytokine that enhances T-cell priming and stimulates fibroblasts, endothelial cells, macrophages, and epithelial cells to produce multiple pro-inflammatory cytokines such as **IL-6, TNF-α**)(Stedman and Byrne, 2012)(Aktunc *et al.*, 2011).

Atorvastatin in high dose significantly decreases **MPO** than in low dose (Rashidian *et al.*, 2016). These results indicate that the inhibition of neutrophil accumulation by

statins may be responsible for protecting AA-induced colonic mucosal injury because **MPO** activity, an index of tissue associated neutrophil accumulation, significantly increased in the colonic mucosa after AA administration (Maheshwari *et al.*, 2015). At last, the effect of statins may be dose-dependent, as 2 studies only found a decrease in histological activity with high dose regimens(Abe *et al.*, 2012)(Cote-Daigneault *et al.*, 2015).

4.4 Effect rosuvastatin(low dose) in acetic acid-induced colitis in mice

Rosuvastatin in low dose attenuated **DIA** by decreasing the sum of weight change, gross bleeding, and stool consistency scores). Mice in the rosuvastatin group showed less **DIA** SCORE than in the AA-induced colitis model (Shin *et al.*, 2017). Statin including rosuvastatin in low dose decrease the colonic **weight/length** ratio compared with AA- induced colitis group. That refers to decrease colonic inflammation (decreased colonic length can reflect colonic inflammation)(Bereswill *et al.*, 2010);(Saber *et al.*, 2021).

Histological scores were significantly decreased in the rosuvastatin-treated group,this drug is exhibited moderate crypt degeneration and inflammatory cell infiltration,Rosuvastatin alleviated Acetic acid induced **histologic** damage by decreasing crypt degeneration and inflammatory cell infiltration (Shin *et al.*, 2017) ,also, treatment with low dose rosuvastatin resulted in smaller erosions with fewer neutrophils than in AA-induce colitis group (Naito *et al.*, 2006). low dose rosuvastatin groups showed displayed a significant decrease in **macroscopic** evaluation score by decreasing inflammation and necrosis in mucosal layers (Saber *et al.*, 2021).

Statin including rosuvastatin also decreases **c-reactive protein** (Needham *et al.*, 2013)(Soliman *et al.*, 2019) the regulatory effect of statins on CRP expression occurs at the transcriptional level (Arnaud *et al.*, 2005). at present, only indirect antagonist

action of statins on **CRP** production has been hypothesized, i.e. consequent to inhibited protein geranyl geranyl transferase, a mechanistically more effective antagonism of the **CRP** activity as exerted by statins should be reported (Skowerski *et al.*, 2020).

Treatment with low dose rosuvastatin decrease pro-inflammatory cytokine**(IL-6And TNF-α**) because rosuvastatin inhibits transcription factors NF-κB activation in the gut, decreased the intestinal production of pro-inflammatory cytokines, and protected the structures of the ileal mucosa(Saber *et al.*, 2021)(Hu *et al.*, 2020).

The present study has shown that **MPO** activity, an index of tissue associated neutrophil accumulation, significantly increases in the colonic mucosa after A-A administration and that this increase is significantly inhibited by treatment with rosuvastatin. These results indicate that the inhibition of neutrophil accumulation by rosuvastatin may be one of the protective factors helping to decrease AA-induced colonic mucosal injury. The inhibition of neutrophil-endothelial interaction by statins has also been reported by other studies (Naito *et al.*, 2006).

4.5 Effect rosuvastatin(high dose) in acetic acid-induced colitis in mice

DAI score was significantly decreased in the mice treated with high dose rosuvastatin than A A group and low dose rosuvastatin**. DAI** score, an indicator of the severity of intestinal inflammation, was a significant increase in the colitis group. In the rosuvastatin-treated groups, we did not observe bloody stools, stools were better formed, and weight loss was lessened (Maheshwari *et al.*, 2015).

The **weight/length** ratio was calculated as the ratio of the colon specimen (mg/cm). It was used as a parameter to assess the degree of colon oedema, which reflected the severity of colitis. treatment with high dose rosuvastatin more reduction **W/L** ratio than in induction and low dose rosuvastatin group that refers to

the high decrease of inflammation and severity of colitis (Mehesen *al.*, 2015) (Naito *et al.*, 2006).

Our results indicated that treatment with RSV dramatically improved the **histological** changes and significantiy reduced the histological score induced by A-A in high dose than in low dose , rosuvastatin was a more reduction of necrosis and inflammation of the epithelial cell (Mehesen *al.*, 2015).
Additionally, these groups showed a significant decrease in **microscopic** evaluation scores (Saber *et al.*, 2021).

Treatment with high dose statin significantly decreases **CRP** than in control and low dose statin (Needham *et al.*, 2013). as well as it plays a direct role in the inflammatory process.**CRP** stimulates monocyte release of inflammatory cytokines such as IL-6 and TNF. In patients treated with statins, a marked decrease in inflammation-associated markers such as the CRP has been described (Ascer *et al.*, 2004). The treatment with 10 mg rosuvastatin in A A-treated group showed a significant decrease in colonic **TNF-α** compared with the low dose rosuvastatin group (Soliman *et al.*, 2019).

Recently, TNF-α has been shown to play a role in the pathogenesis of IBD (Perše and Unkovič, 2020). It was previously reported that the contribution of T-helper 1 responses in the generation of **TNF-α** in the AA-induced colitis in mice. It was also demonstrated that suppression of **TNF-α** by statin, accompanied by considerable inhibition of intestinal inflammation in AA-induced colitis. Therefore, we concluded the effects of rosuvastatin in 10mg on colonic inflammation after induction of colitis. In the present study, we confirmed that the reduction of mucosal **TNF-α** might be responsible for the anti-inflammatory effects of statins (Maheshwari *et al.*, 2015). statins have been reported to reduce serum pro-inflammatory cytokines **(**e.g**., TNF-α, IL-6), C-reactive protein (CRP)**, and T cell and monocyte activation (Karmaus *et al.*, 2019)(Ascer *et al.*, 2004).

MPO activity was significantly reduced in the high dose rosuvastatin group, compared with that in the A A and low dose rosuvastatin group. by high inhibition of neutrophil accumulation by rosuvastatin (Honjo *et al.*, 2002)

4.6 Effect prednisolone in acetic acid-induce colitis in mice

The prednisolone was highly significant decrease **DAI** score by more lowering (weight loss, rectal bleeding and diarrhoea) In the MP group, a loose stool was observe on day 3 and had returned to normal by day 6 (Soyturk *et al.*, 2012).

prednisolone pretreatment resulted in a comparatively smaller ratio (**weight/length**) as compared to colitis mice, which indicates a decrease in inflammation in ulcerative colitis (Ansari *et al.*,2021).

The mean **macroscopic** score was significantly reduced by standard (Prednisolone) treatment was found to more reduce hyperaemia, redness and inflammation at the site of ulceration, however more prominent reduction in colonic ulcers, prednisolone significantly restore these macroscopic injuries produced by the AA (Gupta *et al.*, 2018).

Prednisolone lowered the **histopathological** scores by relieving hyperaemia, cellular infiltration and cell hyperplasia among the treated animals, along with this it decreases ulceration, necrosis and oedema as compared to acetic acid-induced colitis mice .it was found to be effective as standard treatment (Gupta *et al.*, 2018). prednisone was associated with significantly decreased mean **CRP** concentrations post-treatment (Jergens *et al.*, 2010).

The prednisolone treatment was effective for decreasing the levels of pro-inflammatory mediators like **TNF-α** and **IL-6**. The levels of mediators were significantly restored at **TNF-α** and **IL-6** as compared to the UC (Gupta *et al.*, 2015).

Prednisolone was a highly reduced **MPO** activity than the acetic acid colitis group (Antonelli *et al.*, 2008).

Conclusion

1. The HMG COA (Atorvastatin and Rosuvastatin) are a potent anti-inflammatory and anti-oxidant and can be used successfully in treatment of experimentally acetic acid induced colitis in mice.

2. Atorvastatin has the more effective therapeutic role than rosuvastatin and its effect was comparable to sulfasalazine through anti-inflammatory and antioxidant actions .

3. High dose of statin is more effective than low dose of statine in treatment of experimentally acetic acid induced colitis in mice.

4. There is positive correlations between histopathological score with clinical parameters, cytokines (TNF-α and IL-4), C-reactive protein ,marker of oxidative stress(MPO).

Recommendations

1. The "use of atorvastatin and rosuvastatin in clinical trials for the treatment of ulcerative colitis in human beings.
2. Experimental studies on animal models are recommended to test anti-inflammatory effect of tested drugs in other inflammatory settings such oral ulcers, pancreatitis and chronic hepatitis.
3. Further experimental studies are recommended to investigate the effect of combination of prednisolone with each of investigated drugs or combination between tested drugs.
4. Experimental studies are recommended to test other members of drugs or another dose within the same groups on experimentally acetic acid induced colitis in" mice.

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