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Effect of glycyrrhizic acid, ammonium salt in experimental animal models of inflammatory bowel disease

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Abstract

Licorice is known herb applied for the inflammation, gastritis, ulcers, arthritis. Root contains glycyrrhizin used as demulcent in inflammatory affection and irritable conditions of bowel. The most important and well-known bioactive components of licorice root are the triterpene glycoside, Glycyrrhizic Acid and its aglycone, 18β-β glycyrrhetinic acid, are having many biological activity like anti-inflammatory, anti-ulcer, anti-allergic, anti-dote, anti-oxidant, anti-viral and anti-tumor. Glycyrrhizinic acid, ammonium salt (GA), is evaluated in the present reaserch work for it's effect in inflammatory bowel disease (IBD) . The GA (10, 20 and 40 mg/kg/day i.p.) was tried on three different experimental animal models of IBD, which are acetic acid-induced colitis in mice and indomethacin-induced enterocolitis in rats and ethanol induced colitis in mice. Prednisolone was used as the standard drug for comparison. The GA showed significant inhibitory activity against IBD induced in these experimental animal models. The activity was comparable with the standard drug prednisolone. The results of present work suggested that GA has a pharmacological capacity to reduce oxidative stress, as evidenced by the significant decrease in lipid peroxidation measured as MDA levels, improved appetite along with maintenance of nutritional status and significantly increased plasma total protein level of GA pretreated animals, which helps to improve the antioxidant capacity. The effect of GA is due to pre and post treatment to inducer of respective paradigm. Hence GA will be protective as a prophylactic as well as curative drug in treating the conditions of IBD. The results of present study were suggested that GA may possibly acts as antioxidant in preventing the conditions of IBD.

Keywords: IBD, Glycyrrhizinic acid, Oxidative stress, antioxidant.

Introduction

Inflammatory bowel disease (IBD) is a spectrum of chronic idiopathic inflammatory intestinal conditions[1]. It a common and chronic gastrointestinal disorder characterized by intestinal inflammation and mucosal damage[2]. IBD conventionally divided into two major subtypes: ulcerative colitis and Crohn's disease. Ulcerative colitis (UC) is characterized by confluent mucosal inflammation of the colon starting at the anal verge and extending proximally for a variable extent (*e.g.,* proctitis, left-sided colitis, or pancolitis). Crohn's disease (CD), by contrast, is characterized by transmural inflammation of any part of the gastrointestinal tract but most commonly the area adjacent to the ileocecal valve[1]. Both CD and UC are chronic, relapsing inflammatory disorders of obscure origin. These diseases share many common features but have distinctly different clinical manifestations[3]. The incidence rates of UC and CD in the United States are about 11 per 100,000 and 7 per 100,000, respectively[4]. These diseases account for 700,000 physician visits per year and 100,000 hospitalizations per year in the United States[5]. Although IBD has been described as a clinical entity for over 100 years, it's etiology and pathogenesis have not been defined[4]. The true cause of IBD remains unclear; however, hypothesis for these disorders include a combination of genetic abnormalities, chronic infection, environmental factors (bacterial, viral and dietary antigens), autoimmunity and other abnormalities of immune regulatory mechanisms[6].

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Oxidative stress may play an important role in the pathophysiology of inflammatory bowel disease[7]. The net effect of increased production of ROS and decreased antioxidant capacity is a potent unopposed oxidative stress which appears to be a major pathogenic mechanism in IBD[8].

Licorice is known as GI herb applied for the inflammation, gastritis, ulcers, arthritis[9]. It is helpful to heal stomach and duodenal ulcer by forming protective film over mucus membrane[10]. Root contains glycyrrhizin used as demulcent in inflammatory affection and irritable conditions of bowel[11]. The most important and well-known bioactive components of licorice root are the triterpene glycoside, Glycyrrhizic Acid (GA) and its aglycone, 18β -β glycyrrhetinic acid. GA and its aglycone are known by the wide range of biological activity like anti-inflammatory, anti-ulcer, anti-allergic, anti-dote, anti-oxidant, anti-viral and anti-tumor etc. [12] . Glycyrrhizic acid stimulates gastric mucus secretion through effect on prostaglandins which may explain its ulcer healing property [13].

Glycyrrhizinic acid, ammonium salt (GA),(fig.1.1) is evaluated in the present reaserch work for it's effect in inflammatory bowel disease (IBD) . The pharmacokinetic behavior of glycyrrhizin after intravenous (i.v.), oral and intraperitoneal (i.p.) administration was compared in rats by Yamamura *et.al.* The bioavailability (65-90%) of glycyrrhizin after i.p. administration was enhanced dramatically. The i.p. route of administration may thus improve the bioavailability of glycyrrhizin[14]. Glycyrrhizic acid, ammonium salt, has been reported to have anti-inflammatory, antioxidant action. Its anti-radical activity is responsible for its anti-inflammatory action. From these reported activities it can be seen that these drugs may have an effect on inflammatory bowel diseases. Hence present research work was undertaken. The GA was tried on three different experimental animal models of IBD, which are acetic acid-induced colitis in mice and indomethacin-induced enterocolitis in rats and ethanol induced colitis in mice. Prednisolone was used as the standard drug for comparison. Aminosalicylates and glucocorticoids are very commonly used drugs for IBD. Glucocorticoids are given in acute attacks of the disease and offer immediate relief. Aminosalicylates like sulphasalazine, mesalamine are given chronically to maintain remission and prevent relapses. As we are using acute experimental animal models, we decided to use glucocorticoid as the standard drug.

Material and methods

Acute toxicity studies: (Table 2.1) [15]

Test drug: Glycyrrhizic acid, Ammonium salt, was purchased from Sigma Aldrich Pvt. Ltd., USA.

Preparation of aqueous solution of Glycyrrhizic acid, ammonium salt (GA): Stock solution was prepared by dissolving GA in distilled water. From this stock solution dose of 10 mg/kg/day, 20 mg/kg/day and 40 mg/kg/day was given to the animals from respective groups. The dose selection was based on LD_{50} value of GA, reported earlier in previous literatures. Stock solution was prepared freshly on each day of dosing.

Animals: Healthy Wistar rats (200 to 250 gm) and Swiss albino mice (20 to 30 g) of either sex, procured from Yash farms, Pune, Maharashtra, India were used for the study. They were housed in a group of 4-6 per cage at a temperature of 25˚c + 1˚c and relative humidity of 45 to 55% and under standard experimental conditions (12 h light: 12 h dark cycle). The animals had free access to standard pellet rodent diet (Pranav Agro Pvt. Ltd. Sangli) and water ad libitum throughout the study. All the experiments were carried out between 09.00 to 17.00 hrs. The experimental protocol was approved by institutional animal ethical committee of RD's College of Pharmacy, Bhor, Dist. Pune, Maharashtra, India. (Proposal No. RDCOP/IAEC/15/09).

Preparation of Prednisolone solution: A Wyselon tablet (Wyeth pharma Ltd. Goa, India.) containing 5 mg Prednisolone was powder triturated and suspended in 0.5% sodium CMC[16]. From this stock solution relative doses were selected and administered to respective animals. Stock solution was prepared freshly on each day of dosing.

Effect of Glycyrrhizic acid, Ammonium salt, in acetic acid-induced colitis in mice: (prophylactic model) [16]
Swiss Albino mice of either sex (20–30 g) were divided into six groups (n=6) and treated as follows (Table 2.2) On the 11th day animals were sacrificed by cervical dislocation and dissected opened to remove GIT (from stomach to anus). GIT was flushed gently with saline and cut opened. 10 cm long pieces of colon were removed. Isolated tissues were fixed in 10% formalin saline and examined histopathologically (Shirke *et. al.*, 2004). Before scarification, blood was withdrawn by retro-orbital technique for biochemical estimation.

Effect of Glycyrrhizic acid, Ammonium salt, in indomethacin induced enterocolitis in rats: (prophylactic model) [16]**:** Wistar rats of either sex (200–250 g) were divided into six groups (n=6) and treated as follows (table 2.3)On the 11th day animals were sacrificed by cervical dislocation and dissected opened to remove GIT (from

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stomach to anus). GIT was flushed gently with saline and cut opened. Caecum and 10 cm long pieces of ileum were removed. Isolated tissues were fixed in 10% formalin saline and examined histopathologically. Before scarification, blood was withdrawn by retro-orbital technique for biochemical estimation.

Effect of Glycyrrhizic acid, Ammonium salt, in ethanol induced colitis in mice: (curative model) [17]: Albino mice of either sex (20–30 g) were divided into six groups (n=6) and treated as follows (Table 2.4)After 72 hrs of last administration animals were sacrificed by cervical dislocation & colon was separated. Colon was flushed gently with saline. 10 cm long pieces of Colon were removed. Isolated tissues were used for estimation of lipid peroxidation and some part was fixed in 10% formalin saline and examined histopathologically. Before scarification, blood was withdrawn by retro-orbital technique for biochemical estimation.

Evaluation of disease: The symptoms of IBD includes anemia, anorexia, weight loss, decreased serum albumin level, decreased antioxidant capacity and altered alkaline Phosphatase level. Hence in present research work, these parameters were considered for assessment.

The disease induced in experimental animals was evaluated based on its physical, biochemical and microscopic characteristics.

Assessment of colonic lesions: After killing the animals, the colon was rapidly excised, opened and gently rinsed off luminal contents with a solution of sodium chloride. 10 cm of the colon segment was resected distally as much as possible. Weight was taken of this colonic portion. The last 3 cm from from the anus was saved for histological examination[18], the remaining was put in an ice-cold KCl for MDA analysis.

Evaluation based on microscopic (histologic) characters: To process for microscopic studies, 5 µm thick paraffin sections were stained in haematoxyline and eosin. The stained sections were examined for any inflammatory changes like infiltration of the cells, necrotic foci, damage to tissue structures like payers patches, damage to nucleus, etc. This histology was performed in Dr. Dhande's Laboratory, Pune, Maharashtra.

Effect of Glycyrrhizic acid, Ammonium salt, on lipid peroxidation: MDA analysis: The isolated tissues were thawed and washed with 0.9% saline again and cut into small pieces with scissor. Approximately 200-250 mg colonic tissues were then homogenized in 10 volumes (w/v) of ice cold 150 mM KCl using a glass Teflon homogenizer (Remi motors RQ-127A.) for 2 min. at 5000 rpm. The MDA (malondialdehyde) level was measured in this homogenate. Tissue & serum MDA levels were determined by the method of Buege and Aust. 250 µl of serum or tissue homogenate, 500 µl of TBA reagent (3.7 g/L in 0.25 mol/L HCl) and 1.5 ml of 15% trichloroacetic acid (in 0.25 mol/L HCl) were combined in a 10 ml screw-caped tube, mixed and heated for 30 min. in boiling water. After cooling in an ice bath, 3 ml of n-butanol was added, mixed and centrifuged (Remi motors RM- 12C.) and the chromogen extracted. The absorbance of the organic phase was determined spectro-metrically (JASCO instruments V-530.) at 535 nm against a blank[19]. The results were expressed as % lipid peroxidation and compared with control taken as 100 %[20].

Measurement of total protein in plasma: Total protein in plasma was measured by modified Biuret, End point assay method using a diagnostic test kit of Span diagnostics Ltd.

Measurement of Alkaline Phosphatase in serum: Alkaline Phosphatase in serum was measured by method of King and Armstrong, using 4- amino antipyrin[21].

Measurement of hemoglobin: Hemoglobin was measured by method of Sahli (acid hematin) method[22].

Statistical analysis: The arithmetic mean ± SEM values were calculated for each experiment. The results were analyzed by using One-Way ANOVA followed by Dunnet's test. The Statistical analysis was carried out with the help of Graph Pad Prism 5.

Results and Conclusion

Administration of 0.1 ml of 6% acetic acid, 7.5 mg/kg indomethacin and two intrarectal injections of 50% ethanol after starting the GA treatment, shown mucosa with areas of ulceration with inflammatory cell infiltration in the region of ulceration, inflammatory cells include neutrophils, few lymphocytes and macrophages in colitis control as compared to normal control group.GA (20 and 40 mg/kg/day, i.p.) and prednisolone (1.14 mg/kg, p.o.) treatment shown prevention of mucosal ulceration and inflammation. Most of the histopathological changes were minimized and found negligible. But GA 10 mg/kg/day was not found to be effective in preventing histopathological injury of colon. (fig.3.1, 3.2, 3.3)

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Inflammatory bowel disease (IBD) is a localized inflammation of the small and large intestine. IBD is characterized by intestinal inflammation and mucosal damage. In the present study effect of Glycyrrhizic acid, Ammonium salt is evaluated by using acetic acid induced colitis in mice, indomethacin induced enterocolitis in rats and ethanol induced colitis in mice. Epithelial or mucosal necrosis and transient inflammation can be induced by luminal instillation of dilute acetic acid in dose responsive fashion. Initial injury in this model was a relatively bland epithelial necrosis and edema that variably extended into lamina propria, submucosa and external muscle layers, depending on the concentration and length of exposure of acetic acid. Mucosa and sub mucosal inflammation followed initial injury and was associated with activation of arachidonic acid pathways. Acetic acid induced colitis is a easily inducible model for IBD and the similarity of the inflammatory mediators profile to IBD suggest that inflammatory phase bears some resemblance to acute human intestinal inflammation. Treatment with either antioxidant improved the macroscopic and microscopic scores of this model[2]. The mechanism by which acetic acid produces inflammation appear to be involve the entry of the protonated form of the acid into epithelium, where it dissociates to liberate protons within intracellular acidification that most likely accounts for the epithelial injury observed[16].

In the present research work, it was observed that intrarectal instillation of acetic acid in mice affected only the distal colonic portion. These effects were found to be similar as reported by Shirke et.al. They proposed that the inflammation was not transmural. Massive necrosis of mucosal and submucosal layers was observed. This was also observed in the present study. Indomethacin given subcutaneously in rats affected the middle portion of the small intestine (jejunum and proximal ileum) and most severely the caecum. Necrotic foci were transmural. These finding suggest that this experimental model resembles Crohn's disease. Pathogenic mechanism of indomethacin was thought to be prostaglandin related[16]. Initial epithelial damage is mediated partly by synthesis inhibition of the protective prostaglandins PGE1, PGE2 and prostacyclines[2].

The Gastroduodenal toxicity of conventional non-steroidal anti inflammatory drugs (NSAID's) is widely recognized. Direct contact of NSAID's with the gut mucosa plays a major role in the expression of NSAID toxicity. Thus, intestinal toxicity is absent or minimal with the drugs that do not undergo enterohepatic cycling, such as aspirin and nabumetone. Experimental data show that indomethacin binds to bile phosphatidylcholine, thereby transforming mixed micelles into simple bile salt micelle characterized by the stronger detergent effect that results in the damage of the gut mucosa. NSAID induced inhibition of oxidative phosphorylation leads to intracellular ATP deficiency, which in turn causes dysfunction of the tight intracellular junction. This is the main mechanism underlying the increase in the gut mucosa permeability[23]. COX -1 regulates gastrointestinal homeostasis through the synthesis of cytoprotective prostaglandins. In mice, COX -1 has been reported to play a protective role against mucosal injury of the small intestine and the colon. Selective COX -2 inhibitors, which are expected to display systemic anti-inflammatory properties without the anticipated gastrointestinal toxicity[24]. In ethanol induced colitis model, mixed inflammatory reaction is triggered by ethanol. An inflammatory reaction in the colonic mucosa and submucosa characterized by an infiltrate of mono and some polymorphoneuclear cells that formed lymphoid aggregates, disruption of mucosal integrity and some erosive spots on the epithelial surface. Ethanol is potent inhibitor of glutathione synthesis and that glutathione levels in antigen presenting cells are an important factor in the prevalence of TH1 versus TH2 profile of immune response. Ethanol may have other irritant and inflammatory effects that stimulate INF-γ production by local and systemic lymphocytes[17].

A majority of patients with Crohn's disease suffer from significant weight loss due to multiple etiologies, including a reduction of spontaneous food intake (SFI) secondary to anorexia. The pathophysiologic mechanisms remain poorly understood. Cytokines mediate inflammation and also cause anorexia. Peripheral and central IL-1 receptors may be involved in mediating anorexia in inflammatory bowel disease[25].

The results of feed intake in acetic acid induced colitis reveled that decrease in feed intake in colitis control animals was significant as compared to normal control group. Hence, it showed that acetic acid induced colitis have produced anorexia. The treatment with Glycyrrhizic acid, Ammonium salt (GA 10 to 40 mg/kg/day i.p.) was shown significant recovery of anorexia in dose dependent manner. In all the paradigms studied viz. acetic acid induced colitis in mice, indomethacin induced enterocolitis in rats and ethanol induced colitis in mice, significant loss in body weight was observed in colitis control group which is reversed significantly by GA treatment in dose

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dependent manner. Acetic acid administration is also responsible for increase in colon weight (mg/cm) due to colitis induction[26] . In the present study also, there is a significant increasing colonic weight after acetic acid administration in colitis control group which was reduced by pre and post treatment with Glycyrrhizic Acid, Ammonium salt.

Anemia of chronic disease is the second most prevalent after anemia caused by iron deficiency and it can occurs in patients with acute or chronic immune activation. The condition has thus been termed "anemia of inflammation." Anemia of chronic disease is immune driven; cytokines and cells of the reticuloendothelial system induce changes in iron homeostasis, the proliferation of erythroid progenitor cells, the production of erythropoietin, and the life span of red cells, all of which contribute to the pathogenesis of anemia[27].In acetic acid and indomethacin induced model, anemia is produced in colitis control significantly as compared to normal control group which is recovered by GA treatment significantly in dose dependent manner. As albumin is recognized as a strong antioxidant, it could be speculated that patients with a low serum albumin concentration will have a significantly diminished plasma antioxidant capacity due to diminished availability of thiol groups. When hypoalbunemia occurs due to diminished nutritional intake, from either illness or anorexia, the burden of oxidative injury may be increased by diminished intake of exogenous antioxidants, such as ascorbate and tocopherols. Thus, it could be speculated that malnutrition, is related to chronic inflammation[28]. Decrease in albumin is associated with decrease in serum total protein[23]. Also serum total protein is decreased in the disease like Ulcerative colitis[29]. In acetic acid induced model, Plasma total protein level was decreased significantly in colitis control group which is found to be increased significantly after treatment with prednisolone (1.14 mg/kg, p.o.) and GA treated (10 to 40mg/kg/day i.p.) as compared to colitis control. In indomethacin induced enterocolitis and ethanol induced colitis model, Plasma total protein level was increased significantly by GA (20 mg/kg/day, 40 mg/kg/day) and by prednisolone as compared to colitis control.

Although Alkaline Phosphatase (AP) is widely distributed in various organs, high levels of AP activities exist in the intestine. In one study authors have investigated intestinal alkaline Phosphatase activity in mucosal biopsies in patients with inflammatory bowel disease. They found that CD influences APs activity in the intestine, increasing their activity as compared with other IBD, such as UC, in which there is reduced enzyme activity. The data presented indicated that AP activity was significantly higher in the intestine of patients with CD[30].

In the present study also, after acetic acid administration, decrease in serum AP levels was found in colitis control as compared to normal control, which was increased insignificantly, but in dose dependent manner, after pre and post treatment with GA (10 to 40 mg/kg/day i.p.). But indomethacin administration was increased serum AP levels in colitis control as compared to normal control, which was decreased significantly by prednisolone and GA pre and post treatment (40 mg/kg/day i.p.) when compared to colitis control group. IBD is characterized by the involvement of reactive oxygen species (ROS) in tissue damage. Since oxygen radicals have high reactivity and short lifetime, it is difficult to assess the involvement and extent of tissue damage induced by oxygen radicals. Therefore this problem has been bypassed by measuring the effects of radical reactions with biological substance. (i.e. lipid peroxides and / or their products as MDA)[19]. MDA is a three-carbon low molecular weight aldehyde and spontaneous breakdown product of peroxides that can be produced from free radical attack on poly unsaturated fatty acids[31]. The TBARS (Thiobarbituric acid reacting substances) method is commonly used to measure MDA in biological samples[32]. The analysis of MDA by the thiobarbituric acid (TBA) assay has been widely employed over the many years in biological systems for the assessment of lipid peroxidation. It is a spectrophotometric assay, based upon heating of the sample under acidic conditions to form the adduct of MDA-TBA[31]. In acetic acid induced colitis, indomethacin induced enterocolitis and ethanol induced colitis model, increased serum and tissue MDA level indicated the significant lipid peroxidation in colitis control group. This increased lipid peroxidation was found to be decreased significantly by GA at all doses tested and by prednisolone. The results of GA were also comparable to that of prednisolone. In acetic acid induced model GA 20 and 40mg/kg/day dose was shown significant reduction in tissue MDA levels as compared to standard prednisolone group. GA at all doses tested was shown significant reduction in serum MDA level as compared to prednisolone treated group. In ethanol induced colitis model, 20 and 40 mg/kg/day dose have decreased tissue and serum MDA level significantly as compared to standard group. From the methodology used and the results of above all paradigms, it is revealed that, the effect of GA is due to pre and post treatment to inducer of respective paradigm. Hence GA will be protective as a prophylactic as well as curative drug in treating the conditions of IBD.

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The results of present study were suggested that Glycyrrhizic acid, Ammonium salt, may possibly reduces the oxidative stress as evidenced by the significant decrease in lipid peroxidation measured as MDA level after the pre and post treatment with GA. Hence it may possibly acts as antioxidant in preventing the conditions of IBD.

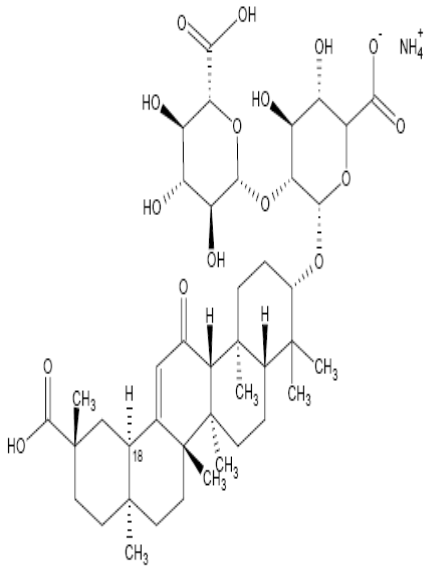

Figure 1.1: Structure of ammonium salt of Glycyrrhizic acid

Table 2.1: Acute toxicities of glycyrrhizin salts in mice

Route	Glycyrrhizin form	LD_{50} (mg/kg)
p.o.	Ammonium, crude	12,700
p.o.	Diammonium	9600
p.o.	Pottassium, crude	12,400
p.o.	Monopottassium	1220
p.o.	Dipottassium	8100
i.p.	Ammonium, crude	1050
i.p.	Monoammonium	1070
i.p.	Diammonium	1250
i.p.	Pottassium, crude	1260
i.p.	Dipottassium	1400
i.v.	Monopottassium	412
i.m.	Monopottassium	695
s.c.	Monopottassium	697

Table 2.2: Effect of Glycyrrhizic acid, Ammonium salt, in acetic acid-induced colitis in mice: (prophylactic model)

Group name	Treatment given	Day of treatment
Normal control	Untreated	Untreated.
Colitis control	0.1 ml of 6% acetic acid	Once intrarectally on 8th day.
GA 1	Test drug (10 mg/kg/day i.p.) and acetic acid	7 days pretreatment and received 0.1 ml of 6% acetic acid solution (once, intrarectally on 8th day); drug treatment continued upto 10 days.
GA 2	Test drug (20 mg/kg/day i.p.) and acetic acid	
GA 3	Test drug (40 mg/kg/day i.p.) and acetic acid.	
Standard	Prednisolone (1.14 mg/kg p.o.) and acetic acid	Prednisolone treatment was started on the day of acetic acid treatment and continued for 3 days.

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Table 2.3: Effect of Glycyrrhizic acid, Ammonium salt, in indomethacin induced enterocolitis in rats: (prophylactic model)

Group name	Treatment given	Day of treatment
Normal control	Untreated	Untreated.
Colitis control	Indomethacin (7.5 mg/kg/day, s.c.)	On two consecutive days (on 8th and 9th day).
GA 1	Test drug (10 mg/kg/day i.p.) and acetic acid	7 days pretreatment and received Indomethacin (7.5 mg/kg s.c.) on two consecutive days (on 8th and 9th day), drug treatment was continued upto 11days.
GA 2	Test drug (20 mg/kg/day i.p.) and Indomethacin	
GA 3	Test drug (40 mg/kg/day i.p.) and Indomethacin	
Standard	Prednisolone (2 mg/kg p.o.) and Indomethacin	Prednisolone treatment was started on the day of indomethacin treatment and continued for 4 days.

Table 2.4: Effect of Glycyrrhizic acid, Ammonium salt, in ethanol induced colitis in mice: (curative model)

Group name	Treatment given	Day of treatment.
Normal control	Untreated	Untreated
Colitis control	200 µl of 50 % ethanol	2 intrarectal administrations 5 days apart (on 3rd and 8th day)
GA 1	Test drug (10 mg/kg/day i.p.) and acetic acid	2 days pretreatment and received 200 µl of 50 % ethanol solution (2 intrarectal administrations 5 days apart; on 3rd and 8th day), drug treatment continued upto 11 days.
GA 2	Test drug (20 mg/kg/day i.p.) and ethanol	
GA 3	Test drug (40 mg/kg/day i.p.) and ethanol	
Standard	Prednisolone (1.14 mg/kg p.o.) and ethanol	Prednisolone treatment was started 8th and continued for 4 days.

Effect of Glycyrrhizic acid, Ammonium salt on physical and biochemical parameters in acetic acid induced colitis in mice:

Table 3.1.1 Physical assessment:

Treatment	Initial body weight (gm)	Final body weight (gm)	Change in body weight	Colon weight (mg/cm)	Feed intake (gm/day)	Diarrhea with blood in stool
Normal control	23.58 ± 0.19	27.10 ± 0.29	3.50± 0.15	84.00 ± 1.65	21.65 ± 0.12	--

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Colitis Control	21.95 ± 0.16	22.97 ± 0.16	1.02 ± 0.087[#]	96.67 ± 1.33[#]	8.34 ± 5.04	+++
Standard	24.32 ± 0.13	27.52 ± 0.32	3.03 ± 0.15[a]	76.67 ± 1.38 [a]	14.97 ± 4.23	--
GA 1	22.38 ± 0.11	23.75 ± 0.09	1.37 ± 0.03	85.33 ± 1.20[a]	10.48 ± 1.37	++
GA 2	21.35 ± 0.10	24.07 ± 0.15	2.71 ± 0.16[a]	81.00 ± 0.89[a]	16.04 ± 0.11	+
GA 3	23.13 ± 0.08	27.47 ± 0.18	4.37 ± 0.15[a]	78.00 ± 1.23[a]	19.09 ± 1.546	--

Values are expressed as mean ± SEM.; [#] p< 0.01 w.r.t. normal control; [a] p< 0.01 w.r.t. colitis control. +++ - severe bleeding in stool with diarrhea; ++ - moderate bleeding in stool with diarrhea; + - less bleeding in stool with diarrhea; -- : neither bleeding nor diarrhea. Standard: Prednisolon 1.14 mg/kg, p.o.; GA 1: 10mg/kg/day i.p.; GA 2: 20mg/kg/day, i.p.; GA 3: 40mg/kg/day, i.p.

Table 3.1.2 Biochemical assessment:

Treatment	Hemoglobin count (gm %)	Plasma Total protein (gm/dl)	Serum ALP (KA Units)	Tissue MDA		Serum MDA	
				% LPO	% inhibition	% LPO	% inhibition
Normal Control	13.33 ± 0.88	7.06 ± 0.42	11.93 ± 0.45	-71.63 ± 3.67	-28.36 ± 3.67	-51.64 ± 5.88	-48.36 ± 5.88
Colitis Control	7.66 ± 0.66[#]	5.43 ± 0.23 [#]	10.12 ± 0.44	100 ± 0.0	00	100 ± 0.0	00
Standard	13.67 ± 0.88[a]	7.52 ± 0.30 [a]	11.96 ± 0.27	64.16 ± 5.07 [a]	35.83 ± 5.07	70.90 ± 3.43 [a]	29.10 ± 3.43
GA 1	8.66 ± 0.33	8.03 ± 0.47 [a]	9.84 ± 0.17	72.06 ± 6.11	27.93 ± 6.11	73.64 ± 4.58	26.36 ± 4.58
GA 2	9.00 ± 0.55	8.80 ± 0.35 [a]	15.98 ± 0.91	41.33 ± 5.60[a,**]	58.67 ± 5.60	54.99 ± 5.39 [a,*]	45.00± 5.39
GA 3	9.33 ± 0.33	9.09 ± 0.58[a]	23.60 ± 1.42	34.91 ± 4.95 [a,**]	65.09 ± 4.95	52.48 ± 4.51 [a,*]	47.51 ± 4.51

Values are expressed as mean ± SEM; [#] p< 0.01 w.r.t normal control; a p< 0.01compared to w.r.t colitis control; * p< 0.05; ** p< 0.01 w.r.t. standard group.
Standard: Prednisolon 1.14 mg/kg, p.o.; GA 1: 10mg/kg/day i.p.; GA 2: 20mg/kg/day, i.p.; GA 3: 40mg/kg/day, i.p.

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Effect of Glycyrrhizic acid, Ammonium salt in indomethacin induced enterocolitis in rats:

Table 3.2.1: Physical assessment:

Treatment	Initial body weight (gm)	Final body weight (gm)	Change in body weight (gm)	Diarrhea with blood in stool
Normal Control	241.3 ± 0.96	294.4 ± 1.83	53.1 ± 0.96	--
Colitis Control	185.2 ± 1.37	196.9 ± 2.22	11.7 ± 0.98#	+++
Standard	242.9 ± 0.828	265.2 ± 1.76	22.3 ± 1.10 b	--
GA 1	227 ±	240.8 ±	13.1 ± 0.58	
GA 2	181.7 ± 0.71	197.5 ± 2.16	15.8 ± 1.60 a	+
GA 3	239.3 ± 1.03	271.9 ± 1.18	32.65 ± 0.44b	--

Values are expressed as mean ± SEM. #p<0.01 w.r.t.normal control; a p<0.05; b p<0.01 w.r.t.colitis control.
Standard: Prednisolon 1.14 mg/kg, p.o.; GA 1: 10mg/kg/day i.p.; GA 2: 20mg/kg/day, i.p.; GA 3: 40mg/kg/day, i.p.
+++ - severe bleeding in stool with diarrhea. ++ - moderate bleeding in stool with diarrhea.
+ - less bleeding in stool with diarrhea. -- - neither bleeding nor diarrhea.

Table 3.2.2 Biochemical assessment:

Treatment	Hemoglobin count (gm%)	Plasma Total protein (gm/dl)	Serum ALP (KA Units)	Tissue MDA -- Ileum		Tissue MDA-Ceacum		Serum MDA	
				% LPO	%Inhibition	% LPO	%Inhibition	% LPO	%Inhibition
Colitis control	11.67+ 0.33 b	5.877 ± 0.16 a	13.06 ± 0.36 a	100 ± 0.0	00	100 ± 0.0 a	00	100 ± 0.0 c	00
Normal Control	14.67 ± 0.33	6.91 ± 0.20	10.62 ± 0.25	-91.45± 0.49	-8.552 ± 0.49	-89.94± 1.52	-10.06 ± 1.52	-79.09 ± 4.01	-20.91 ± 4.01
Standard	13.67 ± 0.88	7.087 ± 0.22*	10.99 ± 0.37 *	85.30± 2.33 *	14.7 ± 2.33	82.32± 3.92**	17.68 ± 3.92	86.33 ±2.64**	13.67 ± 2.64
GA 1	11.33 ± 0.33	6.27 ± 0.14	12.69 ± 0.56	88.83 ± 2.43	11.17 ± 2.43	86.61 ± 2.33 *	13.55 ± 2.33	84.87 ±4.13**	15.13 ± 4.13
GA 2	13.33 ± 0.88	7.08 ± 0.11 **	11.36 ± 0.40	78.28± 3.79**	21.72 ± 3.79	79.68± 4.02**	20.32 ± 4.02	75.44±2.29**	24.56 ± 2.29
GA 3	13.33 ± 0.33	7.553 ± 0.16**	11.01 ± 0.18 *	75.04± 4.94**	24.96 ± 4.94	75.68± 4.56**	24.32 ± 4.56	67.52 ±2.23**	32.48 ± 2.23

Values are expressed as mean ± SEM. a is p<0.05; b is p<0.01; c is p<0.001 w.r.t. normal control;
*is p<0.05; ** is p< 0.01; *** is p<0.001 w.r.t. colitis control group.
Standard: Prednisolon 1.14 mg/kg, p.o.; GA 1: 10mg/kg/day i.p.; GA 2: 20mg/kg/day, i.p.; GA 3: 40mg/kg/day, i.p.

ISSN: 0976-7126

3.3. Effect of Glycyrrhizic acid, Ammonium salt in ethanol induced colitis in mice

Table 3.3.1: Physical assessment:

Treatment	Initial body weight (gm)	Final body weight (gm)	Change in body weight (gm)	Diarrhea with blood in stool
Normal control	24.32 ± 0.20	28.2 ± 0.20	3.88 ± 0.09	--
Colitis Control	26.17 ± 0.16	27.43 ± 0.22	1.27 ± 0.16 #	+++
Standard	26.2 ± 0.21	28.67 ± 0.20	2.47 ± 0.13 a	--
GA 1	22.42 ± 0.17	25.1 ± 0.22	2.68 ± 0.14 a	
GA 2	23.28 ± 0.16	26.15 ± 0.33	2.87 ± 0.21 a	++
GA 3	27.42 ± 0.16	29.97 ± 0.32	2.55 ± 0.16 a	+

Values are expressed as mean ± SEM. # p<0.01w.r.t. normal control; a p<0.01 w.r.t. colitis control.
Standard: Prednisolon 1.14 mg/kg, p.o.; GA 1: 10mg/kg/day i.p.; GA 2: 20mg/kg/day, i.p.; GA 3: 40mg/kg/day, i.p.
+++ - severe bleeding in stool with diarrhea.; ++ - moderate bleeding in stool with diarrhea.
+ - less bleeding in stool with diarrhea.; -- - neither bleeding nor diarrhea.

Table 3.3.2 Biochemical assessment:

Treatment	Hemoglobin Count (gm%)	Plasma Total protein (gm/dl)	Tissue MDA		Serum MDA	
			% LPO	% inhibition	% LPO	% inhibition
Colitis Control	15.00 ± 0.57	6.40 ± 0.27 #	100 ± 0.0	00	100 ± 0.0	00
Normal Control	16.67 ± 0.33	7.73 ± 0.26	-87.49 ± 2.24	-12.51 ± 2.24	-52.12± 1.08	-47.88 ± 1.08
Standard	16.33 ± 0.88	7.85 ± 0.15 b	84.21± 3.22 b	17.59 ± 3.26	41.86 ± 1.16 b	58.14 ± 1.16
GA 1	17.0 ± 0.57	7.31 ± 0.19	88.71 ± 1.65 a	11.29 ± 1.65	83.14 ± 5.24 b,**	16.85 ± 5.24
GA 2	19.33 ± 0.66	7.69 ± 0.30 a	69.98 ± 6.13 b,*	30.02 ± 6.13	62.28 ± 4.67 b,**	37.72 ± 4.67
GA 3	20.0 ± 1.15	7.74 ± 0.240 b	40.40 ± 2.60 b,**	59.6 ± 2.60	46.76± 2.07 b,**	53.24 ± 2.07

Values are expressed as mean ± SEM. #p<0.01 w.r.t. normal control;
a p<0.05; b p<0.01 w.r.t. colitis control.; * is p <0.05; ** is p<0.01 w.r.t. standard group.
Standard: Prednisolone 1.14 mg/kg, p.o.; GA 1: 10mg/kg/day i.p.; GA 2: 20mg/kg/day, i.p.; GA 3: 40mg/kg/day, i.p.

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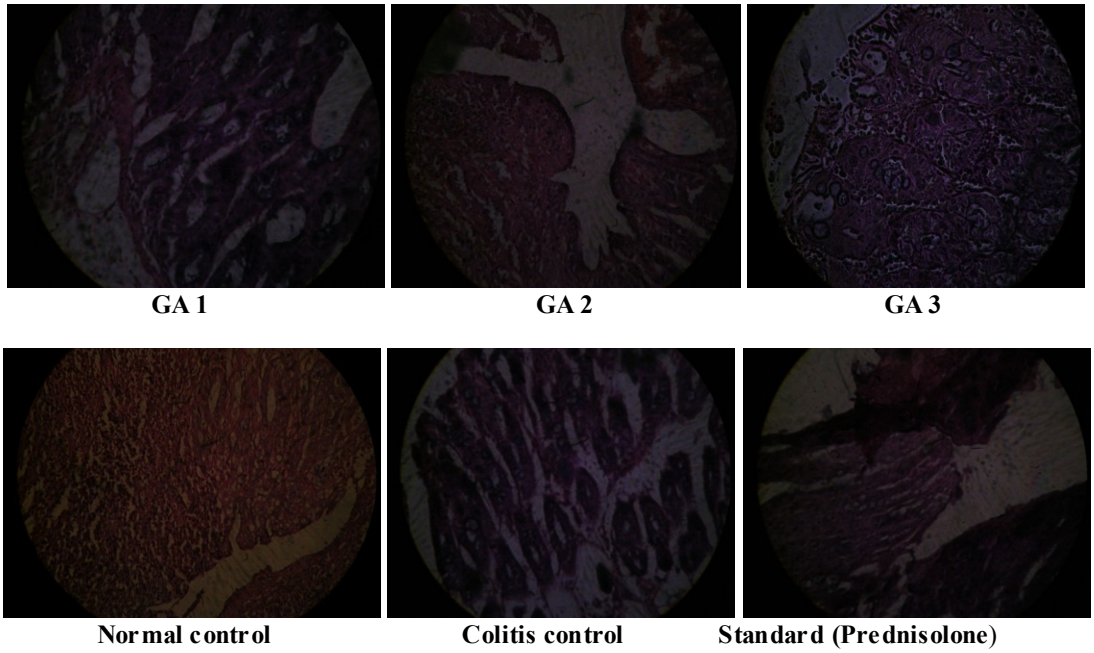

Fig. 3.1: Effect of Glycyrrhizic acid, ammonium salt on histopathology of colon in acetic acid induced colitis in mice

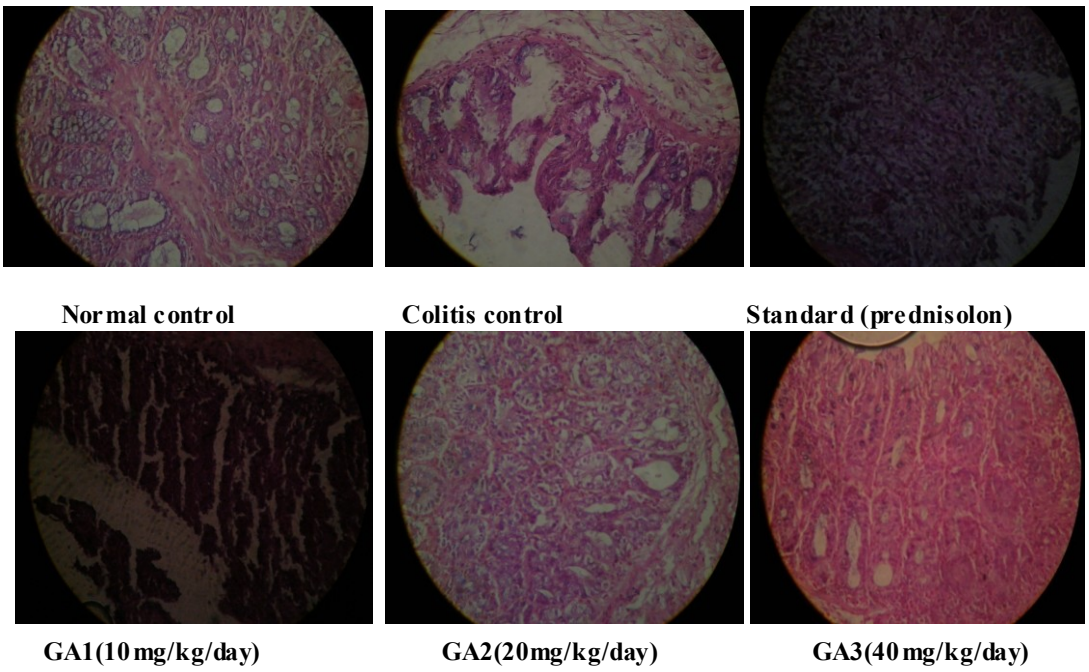

Fig. 3.2: Effect of Glycyrrhizic acid, ammonium salt on histopathology of ceacum in indomethacin induced colitis in rats

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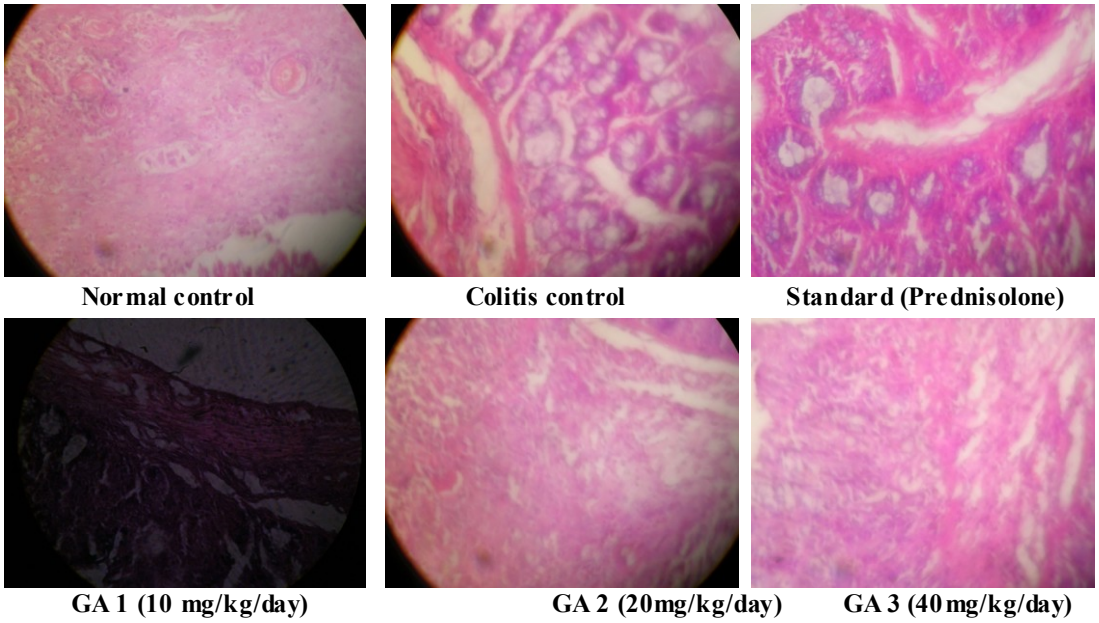

Fig. 3.3: Effect of Glycyrrhizic acid, Ammonium salt on histopathology of colon in ethanol induced colitis in mice

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References

1. Sellin J.H. and Pasricha P.J. (2006). Pharmacotherapy of inflammatory bowel disease. In *Goodman & Gilman's, Pharmacology Basis of Therapeutics* by Brunton *et.al.,* McGraw Hill Medical Publishing Division, New York; 11th edition, 1009.
2. Jurjus A.R., Khoury N.N. and Reimund J.M. (2004). Animal models of inflammatory bowel disease. *Journal of Pharmacological and Toxicological Methods*, **50**: 81-92.
3. Kumar Abbas and Fauseo (2007). *Robbins and Cotran- Pathological Basis of Diseases*, Elsevier publishers, 7th edition, 846.
4. Friedman S. and Blumberge R.S. (2005). *Inflammatory bowel disease in Harrison's Internal Medicines*, McGraw Hill Medical Publishing Division, New York, 16th edition, vol. II, 1776.
5. Achkar J.P. (2008). Inflammatory bowel disease. Review by The American College of Gastroenterology. 163-175.
6. Wall G.C. (2005). *Lower Gastrointestinal Disorders in Applied Therapeutics* by Koda Kimble, Lipponcott William and Wilkins Publishers, 8th edition, 28-1.
7. Narushima S. and Spitz D.R. (2003).Evidence for oxidative stress in NSAID- induced colitis in IL-10 mice. *Free Radical Biology and Medicine*, **34 (9)**: 1153–1166.
8. Kaplan M., Mutlu E.A. and Benson M. (2007). Use of herbal preparations in the treatment of oxidant-mediated inflammatory disorders. *Complementary Therapies in Medicine*, **15**: 207—216.
9. Silver R.J. (2005). The 10 Herbs Your Clients Most Want to Know About. Boulder's Natural Animal, An Integrative Wellness Center Boulder, CO, USA.
10. Roger P. and George D. (2006). *Encyclopedia of Medicinal Plant*. Editorial safeliz S.L. Publishers, vol. 1, 308.
11. Nadkarni K. M., 1982. *Indian Material Medica*, Bombay Popular Prakashan, vol 1, 3rd revised edition, 582.

ISSN: 0976-7126

12. Baltina L.A. (2003). Chemical modification of Glycyrrhizic acid as a route to new bioactive compounds for medicines. *Current Medicinal Chemistry,* **10(2)**: 155-71.
13. Fetrow C.W. and Avila J.R. (1999). *Professional Handbook of Complementory and Alternative Medicines*. Springerhouse Corporation, 393.
14. Yamamura Y., Santa T. and Kotaki H. (1995). Administration-route dependency of absorption of glycyrrhizin in rats: intraperitoneal administration dramatically enhanced bioavailability. *Biological & Pharmaceutical Bulletin*, **18(2)**: 337-41.
15. Isbrucker R.A. and Burdock G.A. (2006). Risk and safety assessment on the consumption of Licorice root (*Glycyrrhiza* sp.), its extract and powder as a food ingredient, with emphasis on the pharmacology and toxicology of glycyrrhizin. *Regulatory Toxicology and Pharmacology*, **46**:167–192.
16. Shirke S.S., Jagtap A.G. and Phadke A.S. (2004). Effect of polyherbal formulation on experimental models of inflammatory bowel diseases. *Journal of Ethnopharmacology,* **90**: 195–204.
17. Andrade M.C. (2003). Ethanol induced colitis prevents oral tolerance induction in mice. *Brazilian Journal of Medical and Biological Research*, **36**: 1227-1232.
18. Hakan Y. (2000). The protective effect of fish oil enema in acetic acid & ethanol induced colitis in rats. *The Turkish Journal of Gastroenterology,* **11(2)**: 155-161.
19. Murat M. M. (2006). The effects of Gingko biloba extact on acetic acid induced colitis in rats. *Turkish Journal of Gastroenterology,* **17(3)**: 177-182.
20. Kulkarni S.K. and Bishnoi M. (2005). Relative Role Of Cyclo-oxygenase 2 (COX 2) inhibitors and Lipoxygenase (LOX) inhibitors in aging induced dementia & oxidative damage. *Animals of Neurosciences,* **1:** 6-11.
21. Rajgopal G. (2005). *Practical Biochemistry for Medical, Dental and Allied courses*. Ahuja Book Company Pvt Ltd., 120.
22. Godkar P.B. (2007). *Textbook of Medical Laboratory Technology*. Bhalani Publishing House, 2nd edition, 726.
23. Thiefin G. (2005). Toxic effects of non steroidal anti-inflammatory drugs on the small bowel, colon and rectum. *Joint Bone Spine,* **72**: 286-294.
24. Medany A.E. (2005). The effects of selective cyclooxygenase-2 inhibitors, celecoxib and rofecoxib, on experimental colitis induced by acetic acid in rats. *European Journal of Pharmacology*, **507**: 291-299.
25. Manjakkollai P. (1996). Mode of food intake reduction in lewis rats with indomethacin-induced ulcerative ileitis. *Physiology & Behavior,* **60(2)**: 381-387.
26. Nosalova V., Ondrejickova O. and Pecivova J. (1999). Effect of histamine H1 antagonist dithiaden on acetic acid-induced colitis in rats. *Physiol. Res.,* **48**:65-72.
27. Weiss G. (2005). Anemia of Chronic Disease, *The New England Journal of Medicine*, **10**, 352.
28. Maruyama Y. (2004). Inflammation & oxidative stress in ESRD- the role of myeloperoxidase. *Journal of Nephrology,* **17**: 72-76.
29. Cella J.V. (1991). *Manual of Laboratory Tests*. A.I.T.B.S. Publishers and Distributors, Delhi, 146.
30. Torres M.I. (2007). A new approach using tissue alkaline Phosphatase histochemistry to identify crohn's disease. *Pathology-Research & Practice,* **203**: 485-487.
31. Tukozkan N. (2006). Measurement of total malondialdehyde in plasma and tissues by High-Performance Liquid Chromatography and thiobarbituric acid assay. *Fırat Tıp Dergisi,* **11(2)**: 88-92.
32. Babb (2004). *Oxis* Research, BIOXYTECH MDA-586™, Spectrophotometric Assay for Malondialdehyde. US Patent No. 5726063, 1-9.

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