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Animal models of inflammatory bowel illness and the effects of glycyrrhizic acid, ammonium salt

john Soosamma

Abstract

Inflammation, stomach ulcers, and arthritis are just some of the conditions that licorice has been shown to help. The demulcent glycyrrhizin found in the root is helpful for inflammatory bowel disease and colitis. Glycyrrhizic acid, a triterpene glycoside, and 18- glycyrrhetinic acid, an aglycone of Glycyrrhizic acid, have the most well-known and significant biological activities in licorice root, including anti-inflammatory, anti-ulcer, anti-allergic, anti-dote, anti-oxidant, anti-viral, and anti-tumor effects. In this study, the effects of glycyrrhizinic acid (GA) ammonium salt on inflammatory bowel disease (IBD) are assessed. The GA was tested on mice with acetic acid-induced colitis, rats with indomethacin-induced enterocolitis, and mice with ethanol-induced colitis at doses of 10, 20, and 40 mg/kg/day i.p. The gold standard for this study was the medication prednisolone. In these experimental animal models, GA exhibited strong inhibitory action against IBD. The efficacy was similar to that of the gold standard medication prednisolone. Evidenced by a significant decrease in lipid pero xidation measured as MDA levels, an increase in appetite and maintenance of nutritional status, and an increase in plasma total protein level of GA pretreated animals, the current work suggests that GA has a pharmacological capacity to reduce oxidative stress. GA's impact comes from its treatment of the paradigm's inducer both before and after the experiment. As a result, GA will serve both preventatively and therapeutically in the management of inflammatory bowel disease. According to the findings of the current research, GA shows promise as an antioxidant for warding off IBD symptoms.

Keywords: Antioxidant, Irritable Bowel Syndrome, Glycyrrhizinic acid, and oxidative stress.

Introduction

Chronic intestinal inflammation that has no known cause is known as inflammatory bowel disease (IBD). It is a chronic inflammatory bowel disease that causes intestinal inflammation and mucosal destruction in a large percentage of the population. 2. The two most common forms of inflammatory bowel disease (IBD) are Crohn's disease and ulcerative colitis. Confluent mucosal inflammation of the colon beginning at the anal margin and progressing proximally for a varied e xtent (e.g., proctitis, left -sided colitis, or pancolitis) is the hallmark of ulcerative colitis (UC). The ileocecal valve region is the most prevalent site of inflammation in Crohn's disease (CD), however the illness may affect any section of the gastrointestinal system. Both Crohn's disease and ulcerative colitis are chronic, recurrent inflammatory diseases with unknown causes.

PG and Research Department of Biochemistry, Rajah Serfoji Govt College, Thanjavur, (TN) - India

India There are numerous similarities between these disorders, despite their varied clinical presentations. 3. In the United States, the incidence rates of UC and CD are around 11 per 100,000 and 7, respectively 4. There are 700,000 annual visits to the doctor and 100,000 annual hospitalizations in the US due to these diseases5. IBD has been recognized as a clinical condition for over a century, .

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 e xp lain 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 Ya mamura *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 but its cause and mechanism of development remain unknown. Genetic abnormalities, chronic infection, environmental factors (bacterial, viral, and nutritional antigens), autoimmunity, and other abnormalities of immune regulatory mechanisms6 are all hypothesized to have a role in the development of inflammatory bowel disease

drugs may have an effect on inflammatory bowel diseases. Hence present research work was undertaken. The GA was tried on three different e xperimental 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 se x, 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 lib itum 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 Triturated prednisolone powder was suspended in sodium CMC16 at a concentration of 0.5%. The stock solution was then used to identify appropriate dosages for individual animals. Each dose's stock solution was made from scratch the day before administration.

The protective role of ammonium glycyrrhizic acid in a mouse model of acetic acid-induced colitis.Sixteen (20-30 g) Swiss Albino mice of either sex were randomly assigned to one of six treatment groups (Table 2.2). On day 11, animals were killed via cervical dislocation and opened for dissection to remove the gastrointestinal tract. The digestive tract was punctured and gently flushed with saline. There were ten centimeter-long chunks of colon taken out. Tissue samples were e xa mined histopathologically after being preserved in 10% formalin saline (Shirke et al., 2004). Retro-orbital blood withdrawal was used for biochemical assessment before scarification.

The prevention model of indomethacin-induced enterocolitis in rats treated with ammonium glycyrrhizic acid. Wistar rats of both sexes (weighing between 200 and 250 g) were split into six treatment groups (n=6) and given the following procedures on day 11 (table 2.3). The animals were then killed by cervical dislocation and their digestive tracts (from the stomach all the way down to the intestines) were removed. The digestive tract was punctured and gently flushed with saline. The caecum and a 10-centimeter-long section of ileum were taken out. Histopathological analysis was performed on the isolated tissues after they had been fixed in 10% formalin saline. Retro-orbital blood withdrawal was used for biochemical assessment before scarification.

Mice given ethanol developed colitis; glycyrrhizic acid (Ammonium salt) ameliorated the symptoms. (theory of treatment) Albino mice (20-30 g) of both sexes were randomly assigned to one of six groups (n=6) and treated as shown in Table 2.4.Animals were slaughtered via cervical dislocation and colon separation after 72 hours after the previous dose. A mild saline flush was used to clean the colon. The colon was cut into pieces about 10 centimeters long. Some of the isolated tissues were fixed in 10% formalin saline and examined histopathologically to determine the extent of lip id peroxidation. Retro-orbital blood withdrawal was used for biochemical assessment before scarification.

Disease evaluation: Inflammatory bowel disease (IBD) symptoms include anemia, anorexia, weight loss, low serum albumin, low antioxidant capacity, and an altered alkaline phosphatase. As a result, these factors were taken into account throughout the current study's evaluation.

Physical, biochemical, and microscopic parameters were used to assess the generated illness in experimental animals.

After the animals were killed, the colon was quickly e xc ised, opened, and the luminal contents were gently washed away with a solution of sodium chloride to evaluate for lesions. The most distal feasible 10 cm of colon was removed. This section of the colon was weighed. The last 3 centimeters of the anus were removed and placed in ice-cold KCl for histological examination18, while the rest was frozen for MDA testing.

Microscopic (histologic) features-based evaluation: Paraffin slices 5 m thick were stained with haematoxyline and eosin for microscopic examinations. Inflammatory alterations such as cell infiltration, necrotic foci, destruction of tissue features including payers patches, nucleus damage, etc. were meticulously examined in the stained sections. Dr. Dhande's lab in Pune, Maharashtra, was responsible for this histology.

Lipid peroxidation and the influence of ammonium glycyrrhizic acid. A look at the MDA: We froze the separated tissues, gave them another wash in 0.9% saline, and then sliced them up using scissors. Then, a glass Teflon homogenizer (Remi motors RQ-127A.) was used to pulverize around 200-250 mg of colonic tissues for 2 minutes at 5000 rpm in 10 volumes (w/v) of ice cold 150 mM KCl. Malondialdehyde (MDA) concentration in this homogenate was evaluated. The Buege and Aust technique was used to calculate tissue and serum MDA levels. To a volume of 1.5 ml of 15% trichloroacetic acid (in methanol), add 250 l of serum or tissue homogenate and 3. 7 g/L of TBA reagent (3. 7 g/L in 0.25 mol/L HCl). A 10 ml screw-capped tube, 0.25 mol/ L HCl, and boiling water were combined and heated for 30 minutes. The chromogen was extracted by adding 3 ml of n-butanol, mixing it well, and centrifuging it (Remi motors RM-12C) in a cold bath. Using a spectrophotometer (JASCO instruments V-530.) and a blank19, we were able to calculate the organic phase's absorbance at 535 nm. The outcomes were reported as a percentage of lipid peroxidation, and the control was set to 100%20.

Total protein in plasma was determined using a diagnostic test kit from Span diagnostics Ltd. using a modified Biuret, End point assay technique.

Serum alkaline phosphatase levels were determined using the 4-aminoantipyrin21 technique developed by King and Armstrong.

Sahli (acid hematin) technique 22 was used to assess hemoglobin levels.

For each test, we determined the mean and standard error of the mean (SEM). One-way analysis of variance (ANOVA) and Dunnett's test were used to evaluate the data. Graph Pad Prism 5 was used for the statistical analysis.

The Ends and the Means

Mucosa with areas of ulceration and in flammatory cell infiltration in the region of ulceration, inflammatory cells including neutrophils, few lymphocytes, and macrophages in colitis control as compared to normal control group after administration of 0.1 ml of 6% acetic acid, 7.5 mg/ kg indomethacin, and two intrarectal in jections of 50% ethanol following initiation of the GA treatment.Preventing mucosal ulceration and inflammation with GA (20 and 40 mg/kg/day, i.p.) and prednisolone (1.14 mg/kg p.o.) therapy. The majority of the histological alterations were downplayed as insignificant. However, GA at a dose of 10 mg/kg/day was not successful in avoiding colon histological damage. (fig.3.1, 3.2, 3.3)

Localized inflammation of the small and large intestines characterizes inflammatory bowel disease (IBD). Inflammation and mucosal injury in the intestine are hallmarks of inflammatory bowel disease. Mice with acetic acid-induced colitis, rats with indomethacin-induced enterocolitis, and mice with ethanol-induced colitis were used to assess Glycyrrhizic acid, Ammonium salt's impact in the current research. In a dose-dependent manner, luminal instillation of diluted acetic acid may cause necrosis or mucosal inflammation of the epithelium or mucosa. In this animal, acetic acid exposure resulted in relatively mild epithelial necrosis and edema that, depending on the dose and duration of the acid's exposure, extended into the lamina propria, submucosa, and exterior muscle layers. Initial in jury was followed by mucosa and sub mucosal inflammation, which was linked to arachidonic acid pathway activation. The similarity between the inflammatory mediators profile in acetic acid-induced colitis and inflammatory bowel disease suggests that the inflammatory phase is comparable to acute human intestinal inflammation and makes this model particularly useful for studying this condition. Both the model's macro- and micro-scores improved after antioxidant treatment2. The protonated form of acetic acid seems to enter epithelium, where it dissociates to release protons inside intracellular acidification that likely accounts for the epithelial injure observed16, and so

causes inflammation.

This study found that in mice, intrarectal instillat ion of acetic acid impacted just the terminal end of the colon. Similar results were seen to those reported by Shirke et al. The researchers hypothesized that the inflammation was localized. The mucosal and submucosal layers showed extensive necrosis. This was also a finding of the current investigation. When administered subcutaneously, indomethacin had the greatest effect on the caecum and the middle of the small intestine (jejunum and proximal ileum) in rats. Transmural necrotic foci were seen. These results provide more evidence that the experimental model closely mimics Crohn's disease. It was previously believed that prostaglandins had a role in indomethacin's pathogenic mechanism16. Synthesis suppression of the defensive prostaglandins PGE1, PGE2, and prostacyclines 2 contributes to the first epithelial injury.

Traditional non-steroidal anti-inflammatory medicines (NSAIDs) are well-known to cause gastrointestinal (GI) side effects. An important factor in the manifestation of NSAID toxicity is direct interaction with the gastrointestinal mucosa. Drugs like aspirin and nabumetone that don't go through enterohepatic cycle don't cause or significantly increase intestinal toxicity. It has been shown experimentally that indomethacin binds to bile phosphatidylcholine, converting the bile into a simple bile salt micelle with a greater detergent impact that damages the gut mucosa. Disruption of the tight intracellular connection results from NSAID-induced suppression of oxidative phosphorylation, which in turn results from a lack of ATP inside the cell. This is the primary mechanism that contributes to the 23 rise in intestinal permeability. The generation of cytoprotective prostaglandins by cyclooxygenase-1 (COX-1) controls gastrointestinal homeostasis.

prostaglandins. In mice, COX -1 has been reported to play a protective role against mucosal in jury of the small intestine and the colon. Selective COX -2 inhibitors, which are expected to display systemic anti-inflammatory properties without the anticipated gastrointestinal toxic ity [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 pres enting 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 etio logies, 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 anore xia 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

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 ret iculoendothelial 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 dimin ished 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 in jury may be increased by diminished intake of e xogenous 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 o xygen radicals have high reactivity and short lifetime, itis difficult to assess the involvement and extent of t issue damage induced by oxygen radicals. Therefore this problem has been bypassed by measuring the effects of radical reactions with biological substance. (i.e . lip id 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 lip id 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 mo del, 20 and40 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.

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.

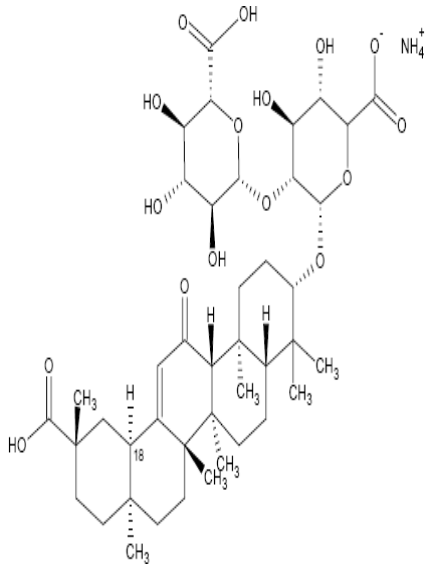

Figure 1.1: Structure of ammonium salt of Glycyrrhizic acid

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.

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

Group name	Treatment gi ven	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 e thanol induced colitis in mice: (curative model)

Group name	Treatment gi ven	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	Col on 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	--

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 ± 0.15a	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 ± 1.20a	10.48 ± 1.37	++
GA 2	21.35 ± 0.10	24.07 ± 0.15	2.71 ± 0.16a	81.00 ± 0.89a	16.04 ± 0.11	+
GA 3	23.13 ± 0.08	27.47 ± 0.18	4.37 ± 0.15a	78.00 ± 1.23a	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 ± 0.88a	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 ± 5.60a,**	58.67 ± 5.60	54.99 ± 5.39 a,*	45.00± 5.39
GA 3	9.33 ± 0.33	9.09 ± 0.58a	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.

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.

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 ± 0.14a	
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± 3.22b	17.59 ± 3.26	41.86 ± 1.16b	58.14 ± 1.16
GA 1	17.0 ± 0.57	7.31 ± 0.19	88.71 ± 1.65a	11.29 ± 1.65	83.14 ± 5.24b,**	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 ± 4.67b,**	37.72 ± 4.67
GA 3	20.0 ± 1.15	7.74 ± 0.240 b	40.40 ± 2.60b,**	59.6 ± 2.60	46.76± 2.07b,**	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.

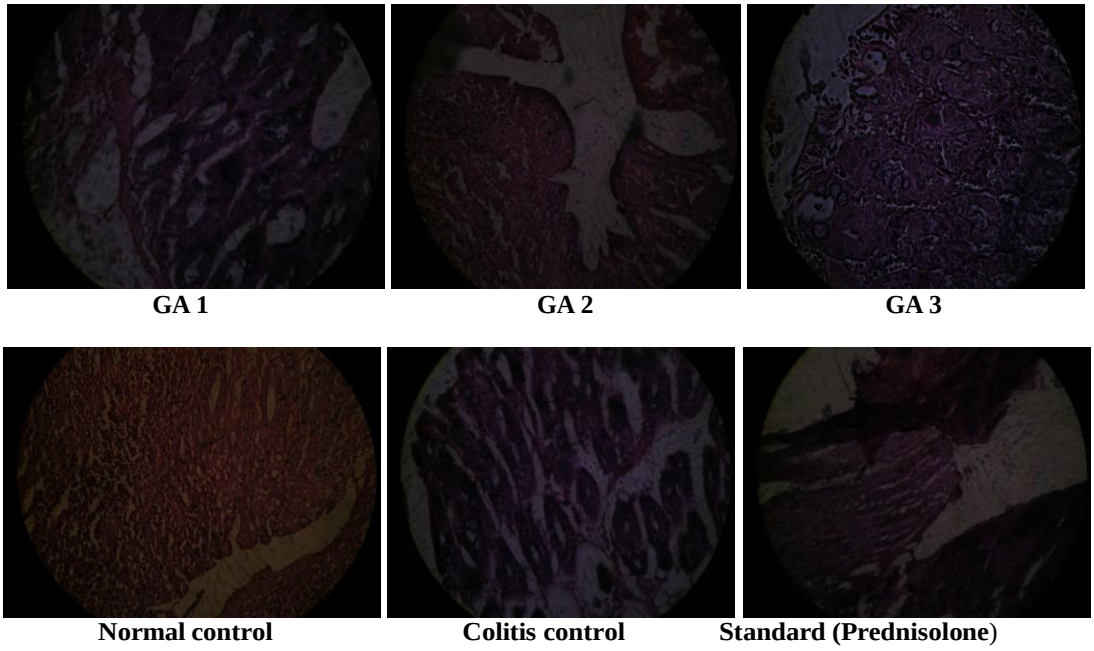

Fig. 3.1: Effect of Glycyrrhizic acid, ammonium salt on histopathology of colon in acetic acid induced colitis in mice

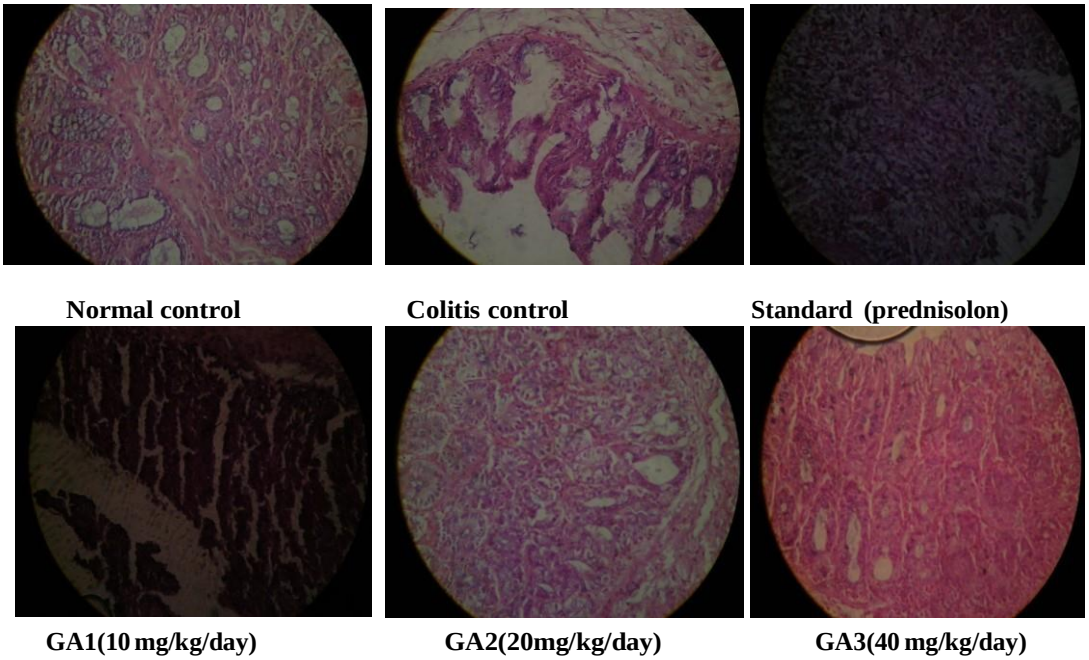

Fig. 3.2: Effect of Glycyrrhizic acid, ammonium salt on histopathology of ceacum in indomethacin induced colitis in rats

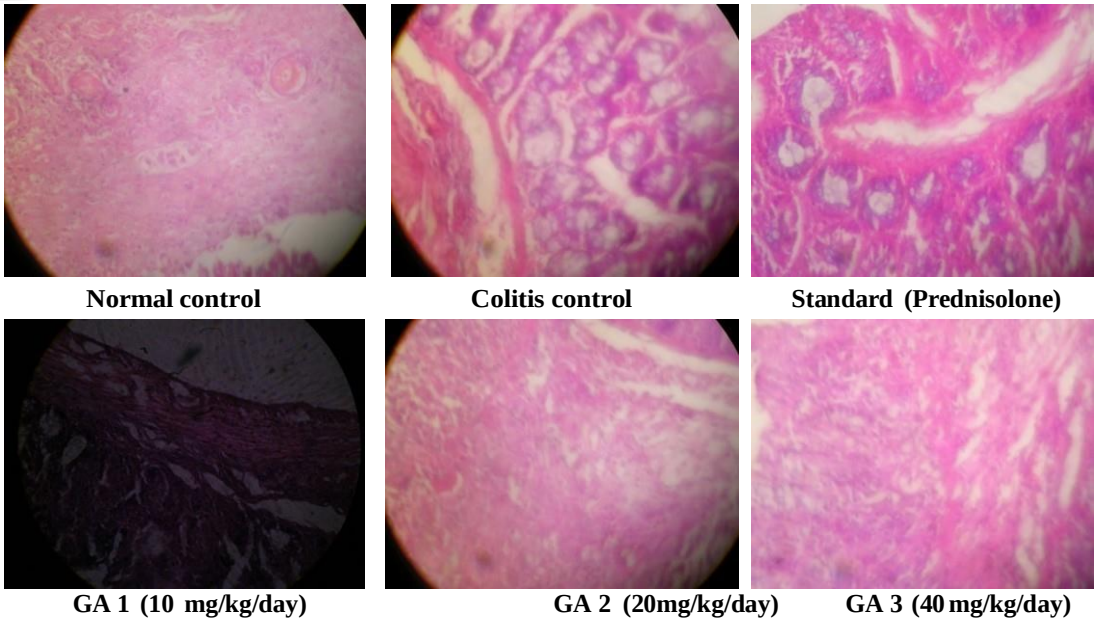

Fig. 3.3: Effect of Glycyrrhizic acid, Ammonium salt on histopathology of colon in ethanol induced colitis in mice

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