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Effect of *Clitoria ternatea* Leaf Extract on TNBS Induced Ulcerative Colitis Rats

Jhansy B*, Somanathan. S. S*.
Department of Pharmacology, Sri Padmavathi School of Pharmacy, Tiruchanoor, Tirupathi.
*Corresponding author's e-mail: drsomu74@gmail.com, jhansy456@gmail.com

Inflammatory bowel disease (IBD) is a chronic intestinal disorder of unknown etiology characterized by chronic and spontaneously relapsing inflammation. Antioxidant, antimicrobial, anti-inflammatory and immuno-modulatory properties of *Clitoria ternatea* suggests that it might exert beneficial effects on inflammatory bowel diseases. Hence the aim of the present study is to investigate the effect of *Clitoria ternatea* leaf extract(CTLE) against 2, 4, 6-Tri Nitro Benzene Sulfonic acid (TNBS) hapten induced ulcerative colitis in rats(UCR). The animals were fasted and made received only vehicle before the induction of colitis. Colitis was induced by intra rectal (*i.r)* application of TNBS (30mg/kg in 0.25ml of 50% ethanol) as a single dose in male wistar rats. The test drug CTLE (200 and 400 mg/kg) and reference standard sulfasalazine (360mg/kg) are prepared in $1^0/_0$ CMC (*p.o*) were administered 6 hours after induction of ulcerative colitis and continued daily for 4 weeks. Normal group receives $1^0/_0$ Carboxy Methyl Cellulose and 0.25ml of phosphate-buffered saline (CMC- PBS-vehicles) in place of TNBS *i.r*. Effects are evaluated on various parameters like wet colon weight/length ratio, tissue damage scores. Superoxide dismutase (SOD), Catalase (CAT), reduced glutathione (GSH) and Malondialdehyde (MDA) content are measured in the colonic tissue homogenate. Intra-rectal instillation of TNBS caused ulcerative colitis with significant decrease in food/water intake, antioxidants levels and loss of body weight. However increased CRP levels and damage scores are observed both macroscopically and histopathologically. CTLE significantly reduced colitis by attenuating the diarrhea, weight loss, macroscopic and histological scores and free radical damage. It showed significant improvement food & water intake and improvement in the levels of SOD, CAT and GSH in dose-dependent manner. Moreover, the effects of CTLE showed as that of the standard. From the above results, it can be concluded that effect of CTLE has a potent therapeutic value in ameliorating colitis induced by TNBS and this effect was more significant with the higher dose (400mg/kg).

Key Words: *Clitoria ternatea,* ulcerative colitis rats, inflammatory bowel disease

INTRODUCTION

Inflammatory bowel disease (IBD) is a chronic inflammatory disorder characterized by up-regulated pro inflammatory mediators and deregulated immune responses resulting in tissue damage.

IBD is of two different types

i) **Ulcerative colitis** (UC affects only the inner lining of the colon)
ii) **Crohn's disease** (Crohn's disease can infect anywhere in the digestive tract, from the mouth to the anus)

The **incidence** of UC varies by time to time and in between different geographic regions throughout the world. Now-a-days prevalence rate was increasing even in developing countries like India (Sood *et al*., 2003; Sivaram Gunisetty *et al*., 2012).

a) Europe
- 0.0243% (Highest incidence)
b) North America
- 0.0192%
c) Asian and Middle East countries
- 0.0063%

It is characterized by the **symptoms** such as bloody diarrhea, abdominal cramping, unintended weight loss and fever (Kucharzik *et al*., 2006; Cho *et al*., 2007). This may be due to infiltration of neutrophils into the colon accompanied by necrosis of epithelial cells and ulceration.

Some studies suggest that multiple immune, genetic and environmental factors influence both the **initiation and progression of colitis** (Strober *et al*., 1998).

Homeostasis of T-helper (Th) Th_1/Th_2 cytokine is important in maintaining intestinal mucosal integrity (Strober *et al*., 2002).

An imbalance between Th_1 and Th_2 cytokines have been implicated in the pathogenesis of IBD.

T-helper cells are the subgroup of lymphocytes, activate and direct other immune cells through release of cytokines [such as interleukin (IL) i.e., IL-1β, IL-6, IL-12, tumor necrosis factor (TNF-α) and interferon (IFN-ϒ)], subsequently leading to macrophage recruitment and activation producing a chronic

inflammation (Inoue *et al*., 1999; Ogata and Hibi, 2003).

Moreover, an increase in Th_2 cytokines was reported in patients with UC, suggesting that UC is a Th_2-mediated.

Conventional drugs, such as corticosteroids and 5-aminosalicylates were effective in the treatment of UC (Bresci *et al*., 1997; Mc Quaid., 2007). But the introduction of immunosuppressive (azathioprine) and biologic agents (TNF blockers) has markedly reduced the need to use corticosteroids for therapy.

This is due to their deleterious side effects and the risk of developing microbial resistance associated with long-term treatment (Navarro & Hanauer, 2003), therefore, researches today are emphasizing on evaluation and characterization of **Herbal constituents** (Polyphenols, triterpenoids, flavonol glycosides, anthocyanins, steroids, antioxidants etc.) against number of diseases based on their traditional uses (Jagtap *et al*., 2004; Yuan *et al*., 2006).

Clitoria ternatea Linn commonly known as 'Butterfly pea', belongs to the family Fabaceae. It is a perennial twinning herb with blue and white flowers. It is distributed throughout India but more naturalized in the tropical regions. It is widely used as nootropic, antistress, antidepressant, anticonvulsant and sedative agent in the traditional system of Indian medicine. Various pharmacological effects like analgesic, anti-pyretic, anti-inflammatory (Parimaladevi *et al*., 2003 and 2004), anti-oxidant (Kamkaen *et al*., 2009), anti-bacterial (Shekawat *et al*., 2010) anxiolytic (Mukherjee *et al*., 2008) and immunomodulatory (Daisy *et al*., 2004) properties of Clitoria *ternatea* Linn suggests that it might be a higher therapeutic potential for IBD conditions.

The objective of present study is to unravel therapeutic potential of *Clitoria ternatea* leaf extract against TNBS hapten induced ulcerative colitis.

MATERIALS AND METHODS

Drugs and chemicals: Sulfasalazine, Trinitrobenzene sulfonic acid (TNBS), Diagnostic kits and other required chemicals were obtained through our institutional store. All other reagents for the estimation of antioxidants were prepared in our institutional lab.

Plant material and preparation of extract: Course powder of the whole plant of *Clitoria ternatea* was procured from Sri Srinivasa Ayurveda Pharmacy, TTD, Srinivasa mangapuram, Tirupati. The powdered material was macerated using hydro-alcoholic (30:70) solvent for a day with occasional shaking at room temperature. It was then filtered, collected introduces in simple distillation under negative pressure then concentrated at 40^0C on a heating mantle until a softy mass obtained. Finally, it was air dried to obtain dry powder extract.

Preliminary Phytochemical screening: The Preliminary phytochemical screening of the hydro alcoholic extract of *Clitoria ternatea* L. (CTLE) was carried out according to the methods described by Khandelwal *et al*.; Kokate *et al*. 27, 28. Phytochemical analysis of the extract was performed for the identification of Phytochemicals like alkaloid, flavonoids, steroids & phenols etc.

Experimental Animals: Healthy male wistar rats weighing (150-200g) were procured from Sri Venkateswara enterprises, Bangalore, Karnataka, India. The animals were then housed as per guidelines of CPCSEA.

Acute toxicity studies: Acute toxicity studiy was performed as per OECD-423 guidelines and the dose was selected as low dose of 200 mg/kg and a high dose of 400 mg/kg.

Induction of ulcerative colitis: Colitis was induced with TNBS as per the technique introduced by **Morris *et al***. Prior to the induction; rats were fasted for 36 hrs with only access to water *ad libitum* and get anesthetized with ether. Then the rats were held in a head down position for 1 min to prevent anal leakage.

Grouping of animals: N=5: N=6 (30 Rats):

Group I : Normal control [0.25ml of Phosphate buffer Saline (*i.r*) + 1% CMC (*p.o*)]

Group II : Disease control [30mg TNBS in 0.25ml of 50 $^0/_0$ ethanol (*i.r*) + 1% CMC (*p.o*)]

Group III : Standard treated [USR + Sulphasalazine-360mg/kg (*p.o*)]

Group IV : Test-1 [USR + CTLE-200mg/kg (*p.o*)]

Group V : Test-2 [USR + CTLE-400 mg/kg (*p.o*)]

Note: The test sample suspension was freshly prepared and administered daily for 4 weeks.

Collection of serum samples: After colitis induction, on day 2 and at the end of the experiment period (i.e., on 29th day), blood was withdrawn from retro-orbital plexus of rat and centrifuged at 2500 rpm for 15 min to collect serum for the estimation of C-Reactive protein using CRP latex slide test.

Assessment of changes in body weight and diarrheal status and mortality: All these parameters observed for each animal daily for 28 days. In addition, the fecal output was scored using arbitrary criteria as follows: **1.** Formed stools **2.** Loosed stools and **4.** Diarrhea (Motavallian-Naeini *et al*., 2012).

Evaluation of physical parameters and macroscopic damage: During study, food and water intake were assessed daily for each group. At the end of the experiment, the number of animals had shown mortality and remaining animals were euthanized by cervical dislocation. The colon (8 cm in length) & spleen specimens were collected and weighed (Drazen Huic *et al*., 2003). Excised colon was opened longitudinally and washed with normal saline solution. The wet colon weight/length ratio was calculated and the macroscopic appearances of the colonic mucosa was scored according to Esmaily and coworkers as follows.

Macroscopic Scoring System of colonic damage: A) Normal appearance with no damage (0); **B)** Localized hyperemia without ulceration (1); **C)** Linear ulceration without significant inflammation(2); **D)** Linear ulceration with inflammation at one site (3); **E)** Two or more sites of ulceration, extending more than 1 cm (4); **F)** Damage extending more than 2 cm along the length of the colon **G)** the score was enhanced by 1 for each increased cm of involvement (5-8). Ulcer area was determined using surgical scale fixed on a light and transparent sheet. Ulcer index was measured by summing the ulcer score and the ulcer area for each colon.

Assessment of colon histological damage: After macroscopic evaluation, colon tissues, 3 cm proximal to the anus was excised and fixed in 10% formalin. Then they were embedded in paraffin, processed, sliced in 4 µm thick sections and stained with haematoxylin and eosin (H&E). Total colitis index (TCI) was calculated by summing inflammation severity, inflammation extent and crypt damage.

Microscopic scoring system of colon damage:

Scoring parameter	Score definition
Inflammation severity	0: None, 1: Mild, 2: Moderate, 3: Severe
Inflammation extent	0: None, 1: Mucosa, 2: Mucosa and submucosa, 3: serosa
Crypt damage	0: None, 1: Basal 1/3 damaged, 2: Basal 2/3 damaged, 3: Crypts lost, surface epithelium present, 4: Crypts lost, surface epithelium lost

BIOCHEMICAL ESTIMATION: The remaining 5 cm colon tissue was homogenized and centrifuged at 10,0000 rpm for 20 minutes to the estimation of colonic mucosal antioxidants like **SOD** (Misra *et al*., 1972), **Catalase**[1], **Glutathione (GSH)** (Moran *et al*., 1998) and free radicals like **Lipid peroxidation** (Malonyl dialdehyde formation) (Slater *et al*., 1971).

Statistical analysis: All the results were expressed as Mean ± SEM. Statistical significance between means of various groups were carried out using one-way ANOVA followed by Dennett's test using computer based fitting program (Graph pad version 6.0) and significance was set accordingly.

RESULTS

Phytochemical screening: CTLE contains Alkaloids, Flavonoids, Phenols, Sterols, Terpenoids, Glycosides and Proteins.

Effect of CTLE and sulphasalazine on TNBS induced changes in body weight and diarrhea

Gr	Body weight (g)			Diarrhea (Scoring index)	
	Initial	Final			
G-i	153.6 ± 8.4	174.0 ± 4.2		1.0 ± 0.0	
G-ii	156.4 ± 1.8	106.2 ± 1.4^{a}	$\downarrow^{sed}$ < G-i	3.6 ± 0.4^{c}	$\uparrow^{sed}$ > G-i
G-iii	151.2 ± 2.4	140.6 ± 1.9^{b}	$\uparrow^{sed}$ > G-ii	2.4 ± 0.4^{d}	$\downarrow^{sed}$ < G-ii
G-iv	151.6 ± 2.4	142.6 ± 4.8^{b}	$\uparrow^{sed}$ > G-ii	2.2 ± 0.4^{d}	$\downarrow^{sed}$ < G-ii
G-v	152.4 ± 2.5	145.6 ± 1.6^{b}	$\uparrow^{sed}$ > G-ii	1.4 ± 0.2^{d}	$\downarrow^{sed}$ < G-ii

All the values are expressed as Mean ± SEM. a= $p<0.0001$ When compared to normal control, b =

p<0.0005 when compared to disease control, c = p<0.01 When compared to normal control, d= p<0.05 when compared to disease control. [Gr / G – Group].

Effect of CTLE and sulphasalazine on TNBS induced changes on food intake and water intake

Gr	Physical parameters	
	Food intake gm/day	Water intake ml/day
G-i	70.4 ± 2.5	214.8 ± 1.4
G-ii	50.0 ± 2.7[a] < G-i	80.0 ± 1.2[c] < G-i
G-iii	58.4 ± 2.5[b] > G-ii	142.0 ± 1.6[d] > G-ii
G-iv	61.2 ± 2.1[b] > G-ii	147.2 ± 1.7[d] > G-ii
G-v	64.6 ± 1.7[b] > G-ii	174.4 ± 0.7[d] > G-ii

All the values are expressed as Mean ± SEM. a= p<0.0001 When compared to normal control, b = p<0.0005 when compared to disease control, c = p<0.01 When compared to normal control, d= p<0.05 when compared to disease control. [Gr / G – Group].

Effects of CTLE and sulphasalazine on the macroscopic parameters of colitis induced by TNBS in rats

Gr	Ulcer score (0-8)	Ulcer area (cm^2)	Ulcer Index	Wet weight of colon/length ratio (mg/cm)	Spleen Index
G-i	0.00 ± 0.00	0.00 ± 0.000	0.00 ± 0.00	36.0 ± 0.60	1.48 ± 0.19
G-ii	6.20 ± 0.20[a]	0.36 ± 0.020[a]	6.56 ± 0.22[a]	109.6 ± 3.70[c]	2.51 ± 0.03[a]
G-iii	3.40 ± 0.40[b]	0.14 ± 0.008[b]	3.54 ± 0.40[b]	71.2 ± 1.35[b]	1.91 ± 0.07[d]
G-iv	3.20 ± 0.37[b]	0.13 ± 0.008[b]	3.33 ± 0.38[b]	67.6 ± 0.90[b]	1.84 ± 0.03[d]
G-v	2.60 ± 0.24[b]	0.09 ± 0.005[b]	2.69 ± 0.25[b]	54.8 ± 1.80[b]	1.55 ± 0.14[d]

All the values are expressed as Mean ± SEM. a= p<0.0001 When compared to normal control, b = p<0.0005 when compared to disease control, c = p<0.01 When compared to normal control, d= p<0.05 when compared to disease control. [Gr / G – Group].

Effects of CTLE and Sulphasalazine on TNBS induced changes in CRP levels

Gr	CRP	
	2nd day	29th day
G-i	9.60 ± 1.47	8.40 ± 1.40
G-ii	38.40 ± 5.80	19.20 ± 2.90[a]
G-iii	33.60 ± 5.80	13.20 ± 2.90[b]
G-iv	38.40 ± 5.80	10.80 ± 1.20[b]
G-v	38.40 ± 5.80	9.60 ± 1.40[b]

All the values are expressed as Mean ± SEM. a= p<0.0001 When compared to normal control, b = p<0.0005 when compared to disease control, c = p<0.01 When compared to normal control, d= p<0.05 when compared to disease control. [Gr / G – Group].

Effects of CTLE and sulphasalazine on TNBS-induced *in-vivo* changes in antioxidants and free radicals

Gr	Anti-oxidants			Free radicals
	SOD (U/mg protein)	CAT (µmoles of H_2O_2/min/mg protein)	GSH (µg/mg protein)	LPO (nmol/mg protein)
G-i	19.42 ± 3.50	24.12 ± 0.10b	10.46 ± 0.53	1.68 ± 0.09
G-ii	4.04 ± 0.55[a]	10.80 ± 0.80[a]	2.94 ± 0.07[a]	3.75 ± 0.30[a]
G-iii	13.54 ± 1.30[b]	17.28 ± 0.70[b]	7.98 ± 0.39[b]	2.18 ± 0.11[b]
G-iv	14.40 ± 2.50[b]	18.00 ± 0.80[b]	8.56 ± 0.79[b]	2.12 ± 0.17[b]
G-v	17.16 ± 1.20[b]	21.20 ± 0.80[b]	9.55 ± 0.03[b]	1.97 ± 0.08[b]

All the values are expressed as Mean ± SEM. a= p<0.0001 When compared to normal control, b = p<0.0005 when compared to disease control, c = p<0.01 When compared to normal control, d= p<0.05 when compared to disease control. [Gr / G – Group].

Effects of CTLE and Sulphasalazine on Microscopic/Histopathological changes of colitis induced by TNBS in rats

Gr	Inflammation severity	Inflammation extent	Crypt damage	TCI
Gr i	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
Gr ii	2.80 ± 0.37[c]	2.40 ± 0.24[c]	3.20 ± 0.20[a]	8.40 ± 0.50[a]
Gr iii	1.20 ± 0.10[d]	1.90 ± 0.10[d]	1.50 ± 0.06[b]	4.60 ± 0.24[b]
Gr iv	1.20 ± 0.12[d]	1.70 ± 0.10[d]	1.30 ± 0.00[b]	4.20 ± 0.20[b]

All the values are expressed as Mean ± SEM. a= p<0.0001 When compared to normal control, b = p<0.0005 when compared to disease control, c = p<0.01 When compared to normal control, d= p<0.05 when compared to disease control. [Gr / G – Group].

Effects of CTLE and sulphasalazine on microscopic/histopathological changes of colitis induced by TNBS in rats (Fig 2)

Histologically, Control group showed increased number of neutrophils and infiltration of leukocytes into mucosal and sub mucosal layers with absence of goblet cells and crypt loss that demonstrates severe inflammation. However, treatments with sulphasalazine and CTLE (200 mg/kg and 400 mg/kg., *p.o*) showed significant decrease in all microscopic parameters (reduced the mucosa injury, minimized the ulceration area) alleviating the colitis by restoring the intestinal cytoarchitecture than Sulphasalazine

Figure 1: Macroscopic presentation of TNBS-induced colitis in rats. **A:** Normal control group with $1^0/_0$ CMC **B:** TNBS (disease control) with $1^0/_0$ CMC. **C:** Colitis treated with Sulphasalazine (standard) suspension. D: Colitis treated with CTLE (Test-200 mg/kg) + $1^0/_0$ CMC. E: Colitis treated with CTLE (Test-400 mg/kg) + $1^0/_0$ CM

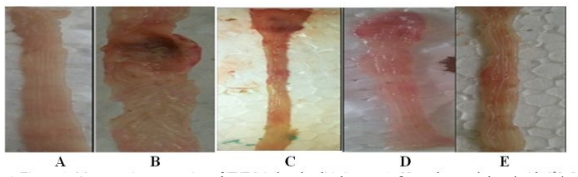
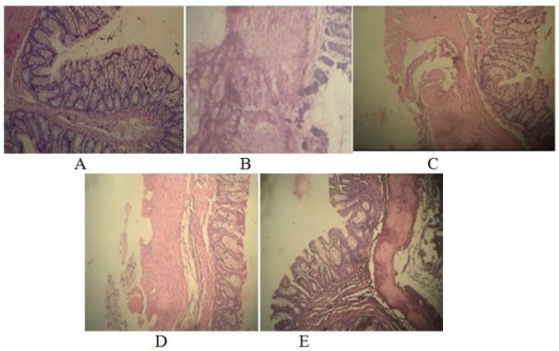


Figure 2: Macroscopic presentation of TNBS-induced colitis in rats. **A:** Normal control group with $1^0/_0$ CMC **B:** TNBS (disease control) with $1^0/_0$ CMC. **C:** Colitis treated with Sulphasalazine(standard) suspension. D: Colitis treated with CTLE (Test-200 mg/kg) + $1^0/_0$ CMC. E: Colitis treated with CTLE (Test-400 mg/kg) + $1^0/_0$ CM.



DISCUSSION

Experimental colitis in rats is useful tool for the investigation of inflammatory reactions in the colon and also for the evaluation of novel anti-inflammatory drugs. In the present study, TNBS has been used as an experimental model to induce colitis because of its clinical features that resembles human IBD (Motavallian-Naeini *et al*., 2012). Additionally, TNBS model can activate both Th_1 &Th_2 (Dohi and Fujihashi., 2006) and induce both acute and chronic phases of colitis depending upon the experiment period (Ajuebor *et al*., 2001).

In the present study rectal administration of 30mg/kg of TNBS (dissolved in 0.25ml of $50^0/_0$ ethanol) induced chronic inflammation in the colonic part of large intestine. This is characterized by intense hyperemia, edema and gut wall thickening assessed by macroscopic scoring and weighing a defined part of the dissected colon (Morris *et al*.,1989).

It is evident from the results that the TNBS control animals experienced a significant body weight loss and increased the diarrhea in comparison with normal group. However the rats treated with CTLE (200 mg/kg and 400 mg/kg, *p.o*) produced a significant increase in body weight and reduced the diarrhea in a dose dependent manner, when compared to TNBS-control group. This might be due to increase in colonic contractility (increase in segmental to and fro movements of the colonic contents) along with its anti-inflammatory (Solanki and Jain., 2012) and immunomodulatory property (Daisy *et al*., 2004).

Alteration in sleeping pattern or exaggeration of postprandial satiety signal from intestine modulates norepinephrine release, through increased release of inflammatory mediators consistent with the slower rate of gastric emptying that makes the animal to have decreased food intake and water intake. Significant decline in food intake and water intake levels was observed in TNBS control group when compared to the normal, whereas the rats received CTLE (200 mg/kg and 400 mg/kg, *p.o*) showed significant improvement in food intake and water intake levels in dose dependent manner providing that it might be due to antihistaminic property of the plant (Youhei Kurose *et al*., 1999; Dnyaneshwar and Ravindra, 2010).

Control group rats showed severe ulceration as evidenced by extensive colonic mucosal and submucosal damage when compared to normal, but the rat groups treated with CTLE (200 mg/kg and 400 mg/kg, *p.o*) significantly decreased all macroscopical damage scores in dose dependent manner. This reason behind this might be inhibition of inflammatory immune responses (i.e., alteration of trafficking of the inflammatory cells *via* modulating expression of chemokines and/or adhesion molecules (Ogata and Hibi, 2003; Yogendrasinh *et al*., 2010).

Increased colon weight/length ratio reflects the degree of local inflammation and decreased capillary permeability was associated with recruitment of macrophages into inflamed tissue producing edema and wall thickening (Parimaladevi *et al*., 2003 and 2004; Shyam kumar *et al*., 2012).The colon weight/length ratio was significantly rised in control animals due to inflammatory response that indicates severity and extent of the disease. However, treatments with CTLE (200 mg/kg and 400 mg/kg, *p.o*) significantly decreased wet weight of colon segments as well as colon damage scores compared to control group animals in dose dependent manner. This may be due to reduced infiltration of inflammatory cells into

inflamed area accounting for the beneficial effect of flavanoids and phenolic compounds etc. against tissue injury (Parimaladevi *et al*., 2004; Niladri Maity *et al*., 2012; Solanki and Jain., 2012)

Spleen, the prerequisite part of the immune system, removes degenerate & aged red blood cells and circulating bacteria from the blood supply. Lesions such as atrophy or fibrosis may occur (directly/indirectly) due to administration of toxic agents causing damage to T and B lymphocytes in spleen (Drazen Huic *et al*., 2003). Diseased control animals showed splenic atrophy, when compared to normal. But the rats received CTLE (200 mg/kg and 400 mg/kg, *p.o*) significantly decreased the splenic enlargement in dose dependent manner, when compared to control. Presence of flavanoids *via* its anti-microbial and immunomodulatory property might be responsible for increase in splenic functioning and decrease in damage to T and B lymphocytes (Daisy *et al*., 2004, Yogendrasinh *et al*., 2010) .

Significant escalation in CRP levels was observed in rats after induction of colitis by TNBS followed by a drop on the 29th day in TNBS control, when compared to normal group. Treatments with CTLE (200 mg/kg and 400 mg/kg, *p.o*) significantly decreased the CRP levels in comparison with TNBS control group animals in dose dependent manner. Probably, this might be due to decreased release of proinflammatory cytokines by its inhibitory effect on humoral antibody formation, phagocytosis, delayed type hypersensitivity response and immune cell activities (Ogata and Hibi, 2003; Daisy *et al*., 2004; Yogendrasinh *et al*., 2010; Shyam kumar *et al*., 2012).

Infiltration of leukocytes into the mucosa has been suggested to contribute significantly to the tissue necrosis and mucosal dysfunction (as they represent a major source of reactive O2 radicals in the inflamed mucosa) in association with colitis. These reactive oxygen species degrade polyunsaturated lipids and forms malondialdehyde. Administration of CTLE (200 and 400 mg/kg) for 28 days significantly decreased the CRP levels as compared to TNBS control group. This might be due to decrease in proinflammatory cytokines associated with TNBS-induced colitis namely IL-1β, IL-6 and TNF-α.

Oxidative stress has been implicated in the pathogenesis of ulcerative colitis in experimental animals (Keshavarzian *et al*., 1990) and in humans (Kitahora *et al*., 1998). Sustained production of reactive oxygen metabolites during colonic inflammation overwhelms the endogenous antioxidant defense system and leads to oxidative injury (D'Odorico, A *et al*., 2001). In the present study, there was a decrease in SOD, CAT, GSH levels after administration of TNBS. But the rat groups those received CTLE (200mg/kg and 400mg/kg, *p.o*) significantly rised SOD, CAT, GSH levels than the rats received TNBS alone, which might be attributed to the antioxidant compounds found in the extract of *Clitoria terneata.*

Infiltration of leukocytes into the mucosa contributes significantly to the tissue necrosis and mucosal dysfunction. In association with colitis, they represents a major source of reactive O_2 radicals in the inflamed mucosa. These reactive oxygen species degrade polyunsaturated lipids and forms malondialdehyde. Increased levels of MDA is used to study the tissue damage *via* lipid peroxidation. In present study, there was an increase in LPO/MDA levels in TNBS control group. But the rats treated with CTLE (200mg/kg and 400mg/kg, p.o) significantly decreased LPO/MDA levels when compared to TNBS control group, which might be due to inhibition of lipid peroxidation by its antioxidant property.

Histologically, the inflammatory response induced by TNBS showed mucosal and submucosal cell infiltration by lymphocytes with alteration of epithelial structure (Morris *et al*., 1989; Julio Gálvez *et al*., 2000) and maldevelopment of goblet cells. Supplementation of CTLE (200mg/kg and 400mg/kg, *p.o*) improved the histological score and attenuated TNBS induced edema formation by virtue of its healing property and this effect was more significant with the higher dose (400mg/kg) administered orally.

In the present study, active constituents obtained from CTLE and its various pharmacological properties might have protected the Intestinal mucosal layers by

reducing the oxidative stress induced cellular damage and also precipitating their microproteins against chemical injuries by its healing property.

CONCLUSION

From the above results, it can be concluded that supplements of CTLE extracts has a potent therapeutic value in ameliorating experimental colitis induced by TNBS and this effect was more significant with the greater dose (400mg/kg) administered orally.

Further scientific studies are necessary to establish the mechanisms involved and the active constituents that were responsible in order to produce therapeutic efficacy against ulcerative colitis.

REFERENCES

1. Aebi H. Catalase in vitro In: SP Colowick, NO Kaplane (eds). *Meth. Enzymol* 1984; 121-26.
2. Ajuebor, M.N., Hogaboam, C.M., Kunkel,S.L., Proudfoot, A. E. I., and Wallace, J. L.. "The chemokine rantes is a crucial mediator of the progression from acute to chronic colitis in the rat," Journal of Immunology 2001; Vol. 166, no. 1, pp. 552–58.
3. Balachandar, B., Jayachitra A., Paramasivam, S and Arulkumar, A. *Alternanthera sessilis* plant extracts using model system for yeast cells. African Journal of Basic & Applied Sciences 2013; 5 (3): 134-38.
4. Bresci, G., Parisi, G., Gambardella, L., Banti, S., Bertoni, M., Rindi, G., Capria, A. Evaluation of clinical patterns in ulcerative colitis: a long-term follow-up. Int. J. Clin. Pharmacol. 1997; Res. 17, 17–22.
5. Cho, J. Y., Chang, H.J., Lee, S.K., Kim, H.J., Hwang, J.K. and Chun, H.S. "Amelioration of dextran sulfate sodium-induced colitis in mice by oral administration of beta-caryophyllene, a sesquiterpene." *Life Sci* 2007; 80 (10): 932-39.
6. Daisy, P., Priya, NN., Rajathi, M. Immunomodulatory activity of *Eugenia jambolana, Clitoria ternatea* and *Phyllanthus emblica* on alloxan-induced diabetic rats, Journal of Experimental Zoology, India, 2004; 7(2), 269-78.
7. Dnyaneshwar, J. Taur., Ravindra, Y. Patil. Pharmacognostic evaluation of *Clitoria ternatea* root. Journal of Pharmacy Research 2010; 3:205-07.
8. D'Odorico, A., Bortolan, S., Cardin, R., D'Inca, R., Martines, D., Fettonato, A., Sturniolo, G.C. Reduced plasma antioxidant concentrations and increased oxidative DNA damage in inflammatory bowel disease. Scand. J. Gastroenterol. 36, 2001; 1289–94.
9. Dohi,T and Fujihashi, K. "Type 1 and 2 T helper cell-mediated colitis," Current Opinion in Gastroenterology 2006, Vol. 22, no. 6, pp. 651–57.
10. Drazen, Huic *et al*. A case report on Splenic atrophy as Complication of Ulcerative colitis diagnosed by antigranulocyte antibodies labeled with Tc-99m-Pertechnetate. *CMJ*. 2003; 44(6):767-69.
11. Du Clos TW, Mold C. The role of C-reactive protein in the resolution of bacterial infection. Curr Opin Infect Dis 2001; 14:289-93
12. Esmaily H, Hosseini-Tabatabaei *et al*. On the benefits of silymarine in murine colitis by improving balance of destructive cytokines and reduction of toxic stress in the bowel cells. Cent Eur J Biol. 2009; 4: 204-13.
13. Inoue, S., Matsumoto, T., Iida, M. Characterization of cytokine expression in the rectal mucosa of ulcerative colitis: correlation with disease activity. Am. J. Gastroenterol. 94, 1999; 2441–46 .
14. Jagtap, AG., Shirke, SS., Phadke, AS. Effect of polyherbal formulation on experimental models of inflammatory bowel diseases. J Ethnopharmacol. 2004; 90:195-204.
15. Julio Gálvez., Margarita Garrido *et al*. Intestinal anti-inflammatory activity of UR-12746, a novel 5-ASA conjugate, on acute and chronic experimental colitis in the rat. Br J Pharmacol.130(8); 2000: 1949-59.
16. Kamkaen, N., Wilkinson, JM., The antioxidant activity of *Clitoria ternatea* flower petal extracts and eye gel, Phytotherapy Research, 2009; 23(11), 1624-25
17. Kucharzik, T., Maaser, C., Lugering, A *et al*. Recent under-standing of IBD pathogenesis: implications for future therapies. Inflamm Bowel Dis 2006; 12:1068-83.
18. Loftus, EV Jr., Sandborn, WJ. Epidemiology of inflammatory bowel disease. Gastroenterol Clin North Am. 2003; 31:1–20.
19. McQuaid, KR. Drugs used in the treatment of gastrointestinal disease. In: Katzung, BG, editor. Basic and clinical pharmacology. 10th ed. New York: McGraw Hill Companies; 2007. p. 1029-35.
20. Misra HP and Fridovich. The role of superoxide anion in the auto oxidation of epinephrine and a simple assay for superoxide dismutase. J. Biol. Chem. 1972; 247, 3170-75.
21. Moran, D., De Buitrago, JMG., Fernandez, E., Galan, AI., Munoz, ME and Jimenez, R. Inhibition of biliary glutathione secretion by cyclosporine A in the rat: possible mechanisms and role in the cholestasis induced by the drug. J. Hepatol 1998; 29: 68-77.
22. Morris, G. P., Beck, P. L., Herridge, M. S., Depew,W. T., Szewczuk, M. R., Wallace, J. L. Hapten-induced model of chronic inflammation and ulceration in the rat colon. Gastroenterology 96, 1989; 795–803.
23. Motavallian-Naeini *et al*. Validation and optimization of experimental colitis induction in rats using 2, 4, 6-trinitrobenzene sulfonic acid. Research in Pharmaceutical Sciences, August 2012; 7(3): 159-69.

24. Mukherjee, P.K., Kumar, V., Kumar, N.S and Heinrich, M. The ayurvedic medicine *Clitoria ternatea* from traditional use to scientific assessment. J. Ethanopharmacol 2008; 120: 291-301.
25. Navarro F & Hanauer SB. Treatment of inflammatory bowel disease: safety and tolerability issues. Am J Gastroenterol 98, 2003; S18–S23.
26. Niladri Maity *et al*. Standardized *Clitoria ternatea* leaf extract as hyaluronidase, elastase and matrix-metalloproteinase-1 inhibitor. Indian J Pharmacol. 2012 Sep-Oct; 44(5):584-87.
27. Ogata, H., Hibi, T. Cytokine and anti-cytokine therapies for inflammatory bowel disease. Curr. Pharm. Des. 9, 2003:1107–13.
28. Parimaladevi B, Boominathan R, Mandal SC, Anti-inflammatory, analgesic and anti-pyretic properties of *Clitoria ternatea* root, Fitoterapia, 2003, 74, 345-49.
29. Parimaladevi, B., Boominthan, R., Mandal, SC. Evaluation of antipyretic potential of *Clitoria ternatea* L extract in rats, Phytomedicine, 2004; 11, 323- 26.
30. Shekawat, M., Vijayvergia, R. Evaluation of Antimicrobial Potential of Some Medicinal Plants against Plants and Human Pathogens, Journal of Pharmacy Research, 2010, 3(4), 700-02.
31. Shyam kumar and Bhat ishwar. Anti-inflammatory, analgesic and phytochemical studies of *Clitoria terneata Linn* flower extract. IRJP. 2012 3(3)
32. Sivaram Gunisetty *et al*. The epidemiology and prevalence of Ulcerative colitis in the South of India. Open Journal of Immunology. 2012; Vol.2, No.4, 144-148. sodium experimental murine colitis. Lab Invest 1993; 69:238-49.
33. Solanki YB and Jain SM. Wound healing activity of *Clitoria terneata L.*in experimental animal models.Pharmacologia 2012; 3(6):160-68,
34. Sood, A., Midha,V., Sood, N., Bhatia,AS., Avasthi,G. Incidence and prevalence of ulcerative colitis in Punjab, North India *Gut* 2003;52:1587-90.
35. Strober, W, Fuss IJ, and Blumberg RS. The immunology of mucosal models of inflammation. Annu Rev Immunol 2002; 20:495–49.
36. Strober, W., Ludviksson, B.R., Fuss, I.J. The pathogenesis of mucosal inflammation in murine models of inflammatory bowel disease and Crohn's disease. Ann. Intern. Med. 1998; 128, 848–56.
37. Vermeire, S., Van Assche, G *et al*. C-reactive protein as a marker for infl ammatory bowel disease. Inflamm Bowel Dis 2004; 10: 661-65.
38. Yogendrasinh B. Solanki., Sunita, M. Jain. Immunomodulatory Activity of Ayurvedic Plant Aparajita (*Clitoria Ternatea L.*) In Male Albino Rats. Global Journal of Science Frontier Research. July 2010; Vol 10, Issue 3 (Ver 1.0), P 2.
39. Youhei Kurose and Yoshiaki Terashima. Histamine regulates food intake through modulating noradrenaline release in the para-ventricular nucleus. Elsevier. Brain research. 1999; Volume 828, Issues 1–2, Pages 115–18.
40. Yuan, G., Wahlqvist, ML., He, G., Yang, M., Li, D. Natural products and anti-inflammatory activity.Asia Pac J Clin Nutr. 2006; 15:143-52.