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RESEARCH ARTICLE

Cytoprotective influence of Ethanolic Stem extract of *Tinospora cardifolia* on the inflammatory bowel disease.

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Abstract

The increased rate of detection of NSAIDS induced small intestinal injury in the past few years could be attributed to notable progress in detection techniques. Development of strategies to assess intestinal injury and measures to be adopted in preventing them therefore assumes greater significance in clinical situations where in, prolonged usage of these drugs is inevitable. The aim of the present study was to carry out pre clinical assessment of *Tinospora cardifolia* for possible protective effect against intestinal damage observed with long term use of anti-inflammatory drug.

Protection against Indomethacin induced damaged of the bowels in rats, was used to carry out the preclinical study. Response of groups of rats that were administered with an ethanolic extract of the stem for a period of ten days were compared with those that did not receive such pretreatment, when challenged with an acute dose of indomethacin during the last two days of the treatment.

Rats were treated with Ethanolic stem extracts *Tinospora cadifolia* (TESs) of both low (250mg/kg) and high doses (500mg/kg) for 10 days by oral route. 5-Amino salicylic acid (5-ASA) standard treatment (10mg/kg) was rendered by oral route for 5days. Indomethacin (IND) at the dose of 10mg/kg was given subcutaneously for all groups except for control group for last two days of treatment period. Severity of ulcers evaluated. Serum myeloperoxidase (MPO), Lactate dehydrogenase (LDH) and tissue associated lipid peroxidase activity were measured. Intestines were subjected for histopathological evaluation.

MPO, LDH, LPO activities and ulcers were elevated in IND group when compared to that of control group. These activities were significantly reduced in TESs group in dose dependent manner with oxidative damage & inflammation.

Tinospora cardifolia was identified as protective agent against Indomethacin induced inflammatory bowel diseases (IBD). This improved integrity of intestine could be attributed to *Tinospora cardifolia*'s anti-inflammatory and anti-ulcerative activities.

Introduction:-

Inflammatory bowel diseases (IBD) comprising Crohn's disease (CD) and ulcerative colitis are well known chronically relapsing inflammatory conditions associated with gastrointestinal tract and are not necessarily

pathogenic in origin (Podolsky et al, 1991). Crohn's disease is a multifocal, transmural inflammatory process that can affect any part of the digestive tract where as Ulcerative colitis is characterized by a superficial, continuous inflammation, which is limited to the large intestine.

Prevalence of IBD appears to be on the increase across different geographic regions of the world. High prevalence rate of CD was reported in Canada (202/100,000 people) and Italy (322/100, 00 people) (Molodecky NA, et al 201), if left unchecked it appears to becoming a burden on public health in coming years. Higher detection rates of enterocolitis made possible because of recent advances in the field of endoscopy, has provided a clearer picture of prevalence. The development has consequently increased awareness of the disease in public domain, medical fraternity, epidemiologists, gastroenterologists and researchers (Yamamoto A, et al 2014, Lim YJ, et al 2013).

Although the precise etiology of IBD still remains obscure, studies carried out in the past decade have unraveled the existence of multiple factors that contribute to its pathogenesis. Genetic predispositions, environmental factors, infectious agents, impairment of local tolerance, and mucosal imbalance with ongoing activation of the intestinal immune system have been implicated (Podolsky et al, 2002).

Advances in the understanding of the underlying immunopathogenetic mechanisms in IBD have resulted in the development of therapeutic strategies that are based on the conception that "uncontrolled activation of central effector cells in the gut is the pivotal pathogenic mechanism involved in the initiation and perpetuation of the inflammatory reaction"(Sartor, R. B . 1994, Neurath , M . F et al 2001). Medications in use to manage the disease are salicylic acid, corticosteroids, immunosuppressive agents, and antibiotics (Podolsky DK 2003). In most cases these therapies offer temporary remission, with no obvious curative effect and enhanced events of adverse reactions (Yanhua X et al 2011) such as psoriasis (Denadai R, et al 2012), drug-induced cytotoxicity (Conklin LS, et al 2012), and hypersensitivity (Sarrazin S, et al 2009) . Search for improved therapy strategies continues.

Prolonged use of Indomethacin is known to induce enterocolitis with pathological features such as transmutable ulcers, wall thickening, adhesions, granulomatous inflammation, crypt abscesses and fibrosis in jejunum-ileum region. The features are similar to those found in Crohn's disease (Sartor RB. 1997, Banarjee A K, et al 1990). Multiple factors namely hepatobiliary export of glucuronides of Indomethacin (Saitta KS, et al 2014, Leite AZ, et al 2001),down regulation of heat shock proteins (Asano T, et al 2009), endoplasmic reticulum stress (Leite AZ, et al 2001) , and deficiency of endogenous prostaglandins (Yokota A, et al 2005) have been identified to be involved in pathogenesis of Indomethacin induced Entrocolites. Increased Oxidative stress, mitochondrial dysfunction and inflammation are found to be prominent factors that cause destructive changes (Leite AZ, et al 2001, Matsui H, et al 2011).

Glucocorticoid-induced tumour necrosis factor receptor (GITR) plays a critical part in regulating both acquired and innate mucosal immune responses during the development of experimental colitis in mice. Therefore, targeting the GITR/GITRL system signalling may represent a potential pharmacological tool for the treatment of IBD (Santucci. L, et al 2007). Human IBD and experimental colitis in mice are associated with immune activation in all GALT organs. Similar to Mesenteric lymph nodes (MLNs), the appendix and the ALF seem to be critical for the development of both IBD and experimental colitis in animals as appendectomy attenuates both conditions (Spahn T W et al 2004).

Tinospora cardifolia commonly known as Guduchi or Amrita belonging to family Menispermaceae is in use in the Ayurveda system of Medicine. It is mentioned in sacred texts Sounakiya Atharvaveda. Charaka identified it as Medharasayana (brain tonic). Sushruta mentioned it in his texts such as Aragvadhadi, Kakolyadi. In Bhavaprakash and Danvantari Nighantu (Sinha K, et al 2004, Kavya B, et al 2015), It has been considered as potent rejuvenator and mentioned for cure of asthma diabetes, jaundice, fever, gout and skin infections (Saha S, et al 2012, Kavya B, et al 2015, Kaur D, et al 2012).

There are reports on extracts of *Tinospora cardifolia* exhibiting potent anti-inflammatory (Saha S, et al 2012), anti-oxidant (Kaur D, et al 2012) and protection against mitochondrial dysfunction (Kosaraju J, et al 2014).
The current study is an attempt to carry out a preliminary investigation of its utility in the treatment of IBD using Indomethacin induced gastric damage in rats as a model for IBD (Colman RW et al 2003, Kaur P, et al 2014). Changes in severity of ulcers, serum myeloperoxidase (MPO), Lactate dehydrogenase (LDH), and tissue associated lipid peroxidase activity and extent of intestinal damage were considered as parameters in the process of evaluation.

Materials and methods:-

Animals:-

Male Wistar rats weighing between 190-240g were used in the study. Animals were acclimatized to an alternate 12h dark-light cycle, temperature of 22±2ºC and humidity of 50±5%, for a week prior to the start of the study. The conditions were maintained untill the end of the study. Feed was provided in the form of pellets that was sourced commercially and water was provided *ad libitum.* Approval for the study in animals was obtained from Institutional Animal Ethics Committee (IAEC/NCP/32/10).

Chemicals:-

Alcoholic extract of Tinospora *cardifolia* stem was procured as a gift sample from Green Chem. pvt.ltd Bangalore. LDH kit (Delta lab, Mumbai), MPO ELISA kit (Aesku Diagnostics, immune shop pvt .Ltd, Mumbai), 2-Thio barbituric acid (TBA) (Sigma fine chemicals), Sodium lauryl sulphate, potassium dihydrogen orthophosphate, Butylated Hydroxy Tolune (BHT) (SD fine Chem Ltd, Mumbai), 5-Amino salicylic acid dissolved in 1% CMC (Quest international) Indomethacin dissolved in 5%$NaHCO_3$ (Sigma Aldrich) were the other kits and chemicals that were sourced from commercial suppliers.

Experimental design:-

Preliminary study was carried out to determine the maximum dose of the extract that did not cause injury leading to death. The experiment was carried out using Wistar rats and the procedures adopted to arrive at the same were in accordance with guidelines set by OECD for carrying out acute toxicity studies (OECD, 2001). A dose of 500 mg/Kg (b.w), of the extract to be administered orally was arrived at as the higher dose that can be used. It was also decided to try a lower dose of 250mg/kg (b.w.) per orally, which represents a geometrical proportional lower dose.

In the study for possible protection against degenerative changes associated with IBD, rats were assigned to form five different groups having six animals each. These groups were identified by numbers assigned to them ranging from1-5. Group I (control) was administered 1% CMC (suspending agent by oral route at a dose of 10 ml/kg b.w. and acted as vehicle control. Group II was administered the lower dose (250mg/kg) of the alcoholic extract of Tinospora *cardifolia, whereas* Group III was administered with the higher dose (500mg/kg b.w). Group IV was administered 5-ASA (10mg/kg) from 5^{th} to 9^{th} day. No drug was administered to the rats in group V.

Except Group I, all other groups were administered Indomethacin (10mg/kg) subcutaneously on the 8^{th} and 9^{th} day so as to induce inflammation of the bowels. On the 10^{th} day animals were sacrificed (Jagtap AG, et al 2011, Kuroda M, et al 2006, Takagi T, et al 2012) and the required body components were taken out to carry out histopathological and biochemical investigation. Procedures were carried out in accordance with CPSCEA guidelines.

Macroscopic evaluation:-

The entire intestine was dissected longitudinally along its anti-mesenteric margin and gross examination of the lumen was carried out using a dissection microscope. The number of bleeding spots, lesions and perforations were recorded. For the assessment of extent of injury a system of grading was used wherein a normal intestinal mucosa was given a score = 0, gentle scattered erosion =1. Diffused intense lesions =2, deep ulcerations, perforations and bleeding =3 (Fukumoto K, et al 2011). Mean score of the group with the associated standard errors, were used for comparison.

Histological evaluation:-

A portion of the intestine of each rat was collected and fixed in 10 % Buffered neutral formalin. Sections were stained with haematoxylin and eosin with slight modification of the method by Bancroft and Stevens (1996). Stained sections were observed for the histopathological changes under light microscope (Nikon, Japan).

Assessment of Myeloperoxidase activity:-

Blood sample were collected from each animal by retro orbital capillary puncture using BD vacutainer tubes. The sample was then centrifuged immediately for 10 minutes at 1000 rpm. Serum was separated and stored at -20ºC. Estimation of Serum Myeloperoxidase was carried out using MPO ELISA kit. Absorbance was read by using Microtiter plate reader at 340nm. The results are expressed in U/ml (Wiess SJ, et al 1982). Mean values obtained for each group were used to carry out comparative studies.

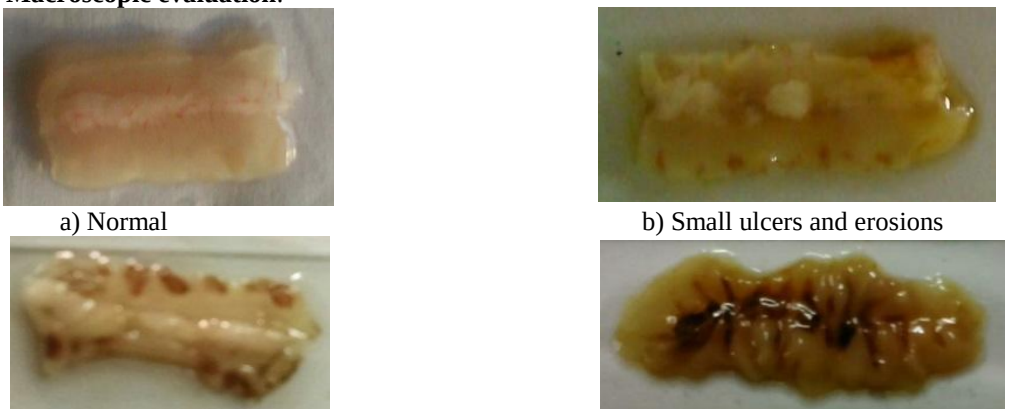
Assessment of Lactate dehydrogenase activity:-
Individual serum samples were analyzed for Lactate dehydrogenase activity using semi auto analyzer (pretiest, Robnik) using LDH kit provided (Delta lab, Mumbai). Absorbance was measured at 340nm and results were expressed in U/l (Magolis SA et al 1977).

Assessment of oxidative stress:-
Oxidative stress was determined using Lipid peroxidation levels in the Intestinal mucosa as an indicator of stress level. The intestinal mucosa was scraped out from each animal and homogenized with potassium phosphate buffer containing KCl and Butylated Hydroxy Toluene. Sodium dodecyl sulphate, acetic acid and Thiobarbituric acid was then added, followed by heating for 60 minutes. To the cooled solution, 5ml mixture of n-butanol and pyridine was added, followed by repeated shaking. Absorbance was measured at 532 nm using a Spectrophotometer and the results were expressed µ mole/mg protein. Mean value obtained for each group was used in comparative studies (Ohkawa et al 1979).

Statistical analysis:-
Descriptive Statistics was made use of in arriving at definitive conclusion using the mean±SEM values observed for parameter. $p<0.05$ was considered as indicator of altered activity. One way ANOVA followed by Dunnett's t test.

Result and Discussion:-

Macroscopic evaluation:



a) Normal b) Small ulcers and erosions

c) Large ulcers, perforations d) hemorrhages, bloody luminal content

Figure - 1: Presence of gentle ulcers, diffused concentrated ulcers, perforations, hemorrhages and bloody lumen in indomethacin induced groups

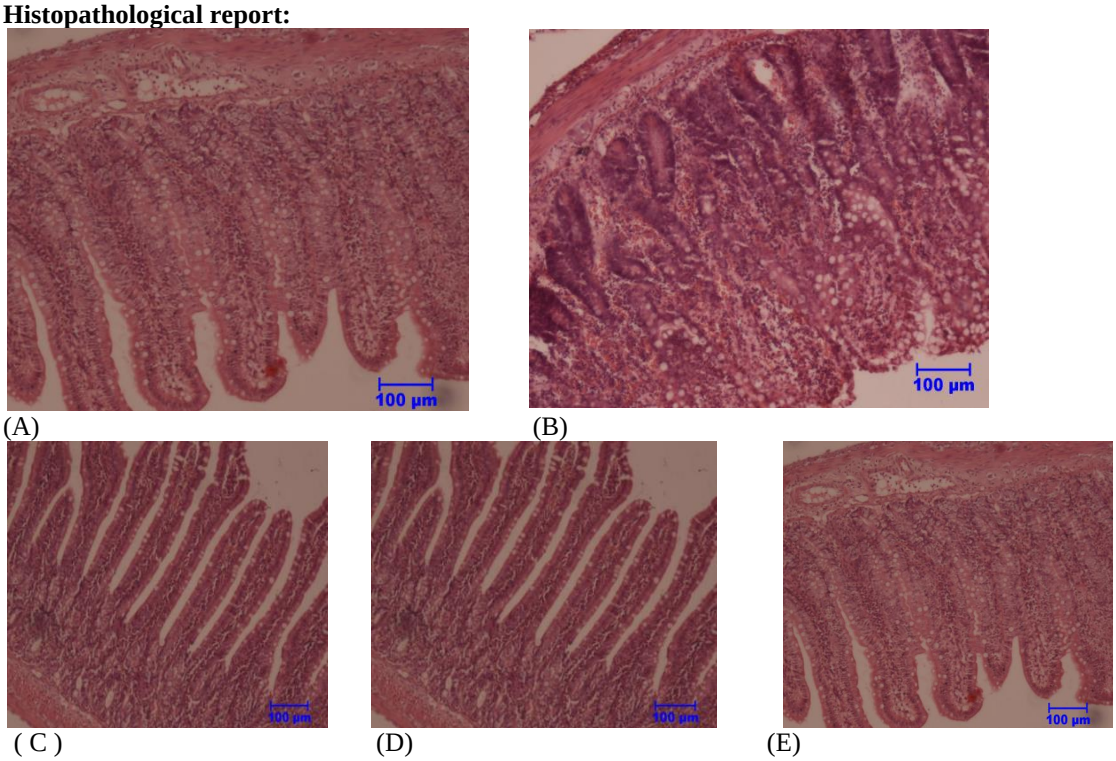
Indomethacin induced intestinal injury was quite evident when observed grossly, ranging from mild injury, perforations to hemorrhage. Haemorrhage was seen in 4 rats of indomethacin group and also in 2 rats of low dose TESs group (Figure 1).High dose TESs group showed very mild injury. TESs attenuated intensity of injury in a dose dependent manner (Table 1).

Table 1: Effect of TESs on Indomethacin induced ulcer scores

Groups	Macroscopic score (MEAN±SEM)
Control	0
Indomethacin(10mg/kg)	2.66±0.21***[a]
Indomethacin+5-ASA(10mg/kg)	1.33±0.21***[b]
Indomethacin+ low doseTESs (250mg/kg)	2.16±0.30**[b]
Indomethacin+high dose TESs (500mg/kg)	0.83±0.16***[b]

Data was analysed using one way ANOVA followed by Dunnett's t test
*p<0.05, **p<0.01, ***P<0.001. n=6,[a] when compared with control group
[b]when compared with indomethacin group. 5-ASA: 5-Amino salicylic acid;
TESs - Ethanolic stem extract of *Tinospora cardifolia.*

Histopathological report:



(A) (B)

(C) (D) (E)

Figure 2: Histological observations in small intestine. A- control group, B- mucosal changes induced by Indomethacin, C- protective effect of standard drug on indomethacin induced injury, D- Effect of low dose TESs (250mg) on indomethacin induced injury, E- Effect of high dose TESs (500mg/kg) on indomethacin induced injury.

In control group, the small intestine of control rats showed normal columnar epithelial cells with goblet cells and sub mucosal layers with cells. The epithelium contains absorptive enterocytes with apical microvilli and many oval, mucous goblet cells were observed. The muscularis mucosa showed lymphocytes in between the muscle layers. Deep in the crypts in the epithelium contains enter endocrine cells with granules in the cell portion facing the lamina propria (figure: 2A).

The Indomethacin given rats showed loss of columnar epithelium with erosion and ulceration, with severe hemorrhages in the sub mucosal layer. There was distortion of intestinal villi architecture, with numerous goblet cells. Infiltrations of inflammatory cells mainly neutrophils (PMN) on the epithelial surface and also in the sub mucosal layers were observed (figure:2B)

The Standard group (5-ASA) rats given standard drug showed regeneration of intestinal mucosal epithelial cells and appearance of goblet cells as that of control rats (figure2C).The rats in low dose TESs group showed recovery from the indomethacin induced erosion, ulceration and regeneration of columnar epithelial cells to a certain extent (figure:2D). High dose TESs group rats showed complete recovery from indomethacin and appear as that of control rat intestine (figure2E).

Table 2: Effect of standard drug (5-Amino salicylic acid) and *Tinospora cardifolia* on indomethacin induced Biochemical changes

Treatment group	**MPO activity (U/ml)± S.E.M**	**LDH activity (U/l) ±S.E.M**	**LPO activity (nmol/mg) ±S.E.M.**
Control	1.88±0.66	264.8±2.482	0.55±0.01
Indomethacin	3.82±0.06***[a]	849.8±16.24***[a]	1.89±0.02***[a]
5-ASA	2.75±0.07***[b]	418±0.774***[b]	0.74±0.009***[b]
TSEs (250mg/kg)	2.38±0.04**[b]	536.8±1.105**[b]	1.04±0.020**[b]
TESs (500mg/kg)	2.58±0.03***[b]	397.3±2.011***[b]	0.843±0.014***[b]

Data was analysed using one way ANOVA followed by Dunnett's t test
*p<0.05, **p<0.01, ***P<0.001. n=6,[a] when compared with control group
[b]when compared with indomethacin group.
5-ASA: 5-Amino salicylic acid; TESs- Ethanolic stem extract of *Tinospora cardifolia.*

Anti-lipid peroxidase activity: Thiobarbituric acid reactive substances in the intestinal mucosa were significantly increased to 1.89±0.02nmol/mg after indomethacin administration. TESs at the dose of 250mg/kg decreased LPO activity. TESs at the dose of 500mg/kg was as efficient as that of standard treatment (5-ASA) in attenuating LPO activity to 0.843±0.014 nmol/mg (Table2).

Anti-myeloperoxidase activity: Indomethacin at the dose of 10mg/kg s.c has mediated increase in MPO activity to 3.82 ± 0.06U/ml. TESs in a dose dependent manner significantly decreased MPO levels and at its high dose(500mg/kg)has notably lessen myeloperoxidase activity to 2.58±0.03U/ml(Table2).

Anti-LDH activity: Lactate dehydrogenase is marker for cell injury. TESs at his higher dose (500mg/kg) inhibited indomethacin induced raise in LDH activity. This was evident by decrease in LDH levels from 849.8±16.24 to 397.3 ± 2.011 U/l (Table2).

Discussion:-

Ulcerative colitis (UC) and Crohn's disease (CD), collectively known as inflammatory bowel disease (IBD), are inflammatory diseases of the enteric mucosa whose etiology is currently undefined. While hypotheses for causation include genetic susceptibility, environmental risk factors, inappropriate and chronic immune responses to members of the intestinal microflora as well as failed immune regulatory mechanisms, many questions regarding IBD susceptibility, dysbiosis and immune dysfunction remain. Not surprisingly, current therapeutic targets are expensive, have questionable long term efficacy and are associated with risk of secondary bacterial and viral infections. As chronically ill patients substitute or supplement their treatments with alternative, nutraceutical remedies in ever greater numbers, more research is needed to ascertain the safety and efficacy of these products as therapeutics.

One of the novel experiments has identified new candidates for anti-colitic therapy or therapeutic supplementation of current IBD treatment strategies. *P. vulgaris, H. gentianoides and E. angustifolia* merit further research as nutraceutical treatments of chronic inflammatory disorders and these studies make it clear that synergy between phenolics, flavonoids and natural anti-bacterial compounds also warrant further research (Kelley Marie Kemper Haarberg 2014).

Research in Inflammatory bowel disease has unraveled pathophysiological pathways and therapeutic strategies. Yet, these findings remain unsatisfactory. Furthermore, conventional treatment used in crohn's disease is not promising and adverse effects of drugs used in management of IBD is reported to cause severe adverse effects such as immune suppression, glucose intolerance, non-hodkins lymphoma, osteoporosis, malignancy, neurotoxicity etc. These findings demands more research to discover novel and potent therapeutics (Triantafillidis JK, et al 2011, Sales H, et al 2015).

The complex tissue problem has riddled IBD research since the emergence of whole genome gene expression analysis. By combining laser microdissected (LM) with new sequencing technology, enabling the identification of cell-type specific differential expression on a whole genome-scale. This opens for a wider understanding of the role of the epithelial monolayer in immune modulation. The identification of a broad over-expression of MHC class I and II genes in IBD epithelium emphasizes the importance of colonic epithelium as antigen presenting cells during inflammation, possibly through TLR3-mediated signaling (Atle Granlund et al 2016).

Inflammation, oxidative stress and mitochondrial dysfunction are found to play pivotal role in pathogenesis of indomethacin induced IBD. TNFα is found to be up-regulating pro inflammatory mediators causing inflammations and manifesting injury in the intestine, Hemopexin is reported to be up regulated in indomethacin induced IBD diseased animal models and being explored as new therapeutic target (Takagi T, et al 2012, Fukumoto K, et al 2011).

However, researchers have also investigated role of oxidative stress in IBD patients and reports have shown abnormal levels of Reactive oxygen species (Iahihara T, et al 2009, Seril DN, et al 2003). In the context of mitochondrial dysfunction and its consequences, it is hypothesized that impairment of activity of respiratory complexes or decrease in complex1 function may release electrons from electron transport chain and contributes to superoxide radical formation. This eventually results in cell injury (Sudeesh NP et al 2013, Chan SH, et al 2009).

Accumulating evidence has indicated the implication of angiotensin II in the pathogenesis of inflammatory bowel diseases (IBD) via its proinflammatory features. Telmisartan (TLM) is an angiotensin II receptor antagonist with marked anti-inflammatory and anti-oxidant actions that mediated its cardio, reno and hepatoprotective actions. With respect to apoptosis, TLM downregulated the increased mRNA, protein expression and activity of caspase3. It also suppressed the elevation of cytochrome c and Bax mRNA besides the upregulation of Bcl2. Together, these findings highlight evidences for the beneficial effects of TLM in IBD which are mediated through modulation of colonic inflammation, oxidative stress and apoptosis (Hany H. Arab et al 2014).

Tinospora cardifolia is used traditionally as potent anti-oxidant, anti-inflammatory agent. Animal studies have demonstrated its protective activity against mitochondrial dysfunction. Furthermore, *Tinospora cardifolia* is found to ameliorate gastric ulcers and injury. These potential properties of drug pushed us to take up this work to evaluate its efficacy against indomethacin induced crohn's disease.

In our study, macroscopic and microscopic evaluation showed protective activity of TESs against injury. One of the approaches to assess this protective activity was to perform LPO and LDH assay to know the extent of protection quantitatively against tissue injury. To get clarity of this protective strategy, MPO assay was employed to know the extent of inflammation.

All these biochemical characters were decreased by TESs. These findings not only contribute to develop TESs as alternative strategy in management of IBD, but also in understanding the involvement of oxidant stress, inflammation in pathogenesis of indomethacin induced IBD (Sinha K, et al 2004, Saha S, et al 2012, Kosaraju J, et al 2014, Kavya B, et al 2015, Kaur D, et al 2012).

Phytochemical screening of aqueous extract of *B. malabaricum* (AEBM) on indomethacin and iodoacetamide induced colitis in rats showed reduction in edema of the intestinal tissues, ulcer protection and lowering of MPO activity in a dose dependent manner. AEBM (500 mg/kg) significantly reduced colonic and serum TNF- α level when compared with the positive control in acetic acid induced colitis model. The results suggest a protective role of AEBM in IBD (Jagtap A G, et al 2011). Same results were compatible in this protective effect of *Tinospora cardifolia* against indomethacin induced IBD too. Therefore, the forthcoming challenge lies not only in finding new targets for novel therapeutic strategies that are based on a sound pathophysiological rationale, but also to find reliable biomarkers that select the most suitable subgroup of patients for each agent. Therefore, a customized therapeutic approach for each individual would truly enable more effective and less toxic treatment of IBD patients (Atreya R, et al 2008).

Pure triterpenoids from apple peel exhibit anti-inflammatory effects by primarily acting on IP-10 gene expression, which plays an important role in inflammation and IBD's, concluded the researchers (Mueller. D et al 2013).

Manifestation of disease by indomethacin induction was done in two days in our study. On the other hand, it may take certain period of time in real practice. Nevertheless, considering indomethacin dose (7.5mg/kg B.W) given subcutaneously in this model and conversion of this animal dose to Human equivalent dose (HED) projects calculated HED as Toxic dose to humans. Even at this toxic dose induction, *Tinospora cardifolia* at its moderate dose (500 mg/kg) has exhibited significant protective activity against injury. Thus, this work could also be utilized in designing adjuvant therapy in indomethacin treatment

Conclusion:-

This study confirms antioxidant, anti-inflammatory potential of alcoholic stem extract of *Tinospora cardifolia*, on the pathological feature in indomethacin induced inflammatory bowel disease. In addition, TESs has also shown to suppress the lactate dehydrogenase activity, which is marker for cell injury. Macroscopic observations confirmed the Histological profile of intestines with mucosal restoration. Hence, Cytoprotective influence of ethanolic Stem extract of *Tinospora cardifolia* were confirmed on the inflammatory bowel disease.

References:-

1. **Asano T, Ichiro K, Yamakawa N, Adachi H, Sobue G, Goto H. (2009).** HSP70 confers protection against indomethacin induced – Protection lesions of the small intestine. J Pharmacol Exp Ther. 330:458-67.
2. **Atle Granlund , Arnar Flatberg, Ann Østvik, Ingunn Bakke, Torunn Bruland, Arne Sandvik. (March 2016).** P-173 Antigen Presentation Activity of the Intestinal Epithelial Cells. Journal of Crohn s and Colitis. - Volume 22 Inflammatory Bowel Diseases: Basic Science Poster Presentations: (C) Crohn's & Colitis Foundation of America, Inc. doi: 10.1097/01.MIB.0000480300.78972.da
3. **Atreya R and Neurath M.F. (May 2008).** New therapeutic strategies for treatment of inflammatory bowel disease Mucosal Immunology, Vol 1; No.3: 175- 182
4. **Banarjee A K, Peters TJ. (1990).** Experimental non-steroidal anti-inflammatory drug-induced enteropathy in the rat: similarities to inflammatory bowel disease and effect of thromboxane synthetase inhibitors. Gut; 31:1358-64.
5. **Chan SH, Wu KLH, Chang AYW, Tai M, Chan JYH. (2009).** Oxidative impairment of mitochondrial electron chain complexes in rostral ventrolateral medulla contributes to neurogenic hypertension. Circulation.53:217-27.
6. **Colman RW, Stadnicki A. (2003).** Experimental models of inflammatory bowel disease. Arch Immunol Ther Exp (Warsz). 51:149-55.
7. **Conklin LS, Cuffari C, Okazaki T, Miao Y, Saatian B, Chen TE. (2012).** 6-Mercaptopurine transport in human lymphocytes: correlation with drug-induced cytotoxicity. J Dig Dis.13:82–93.
8. **Denadai R, Teixeira FV, Saad-Hossne R. (2012).** The onset of psoriasis during the treatment of inflammatory bowel diseases with infliximab: should biological therapy be suspended? Arq Gastroenterol. 49:172–6.
9. **Fukumoto K, Yujinaito. (2011).** Role of TNF-alpha in the pathogenesis of indomethacin induced small intestinal injury in mice. Int J Mol Med. 27:353-9
10. **Hany H. Arab, Muhammad Y. Al-Shorbagy, Dalaal M. Abdallah , Noha N. Nassar. (May 2014).** Telmisartan Attenuates Colon Inflammation, Oxidative Perturbations and Apoptosis in a Rat Model of Experimental Inflammatory Bowel Disease. PLOS ONE; Volume 9. Issue 5: 1- 16. e97193.
11. **Iahihara T, Tanaka K, Taska Y, Namba T, Suzuki J. (2009)** Therapeutic effect of lecithinized superoxide dismutase against colitis. J Pharmacol Exp Ther. 328:152-164.
12. **Jagtap A G, Niphadkar P V, Phadke A S**. **(May 2011).** Protective effect of aqueous extract of *Bombax malabaricum* DC on experimental models of inflammatory bowel disease in rats and mice. Indian Journal of Experimental Biology; Vol. 49: pp. 343-351
13. **Kaur P, Singh K. (2014).** A review on chemical-induced inflammatory bowel disease in rodents. Korean J Physiol Pharmacol; 18:279-88.
14. **Kavya B, Kavya N, Ramarao V, Venkateswarulu G. (2015).** *Tinospora cardifolia* (wild) miers: Nutritional, Ethno medical and Therapeutic utility. Int, J. Res. Ayurveda pharm; 6(2):195-8.
15. **Kaur D, Kumar S, Sharma R, Rana AC. (2012).** Protective effects of *Tinospora cardifolia* against Reserpine induced ulcer model. IOSR J Pharm BiolSci. 3(8):275-9.
16. **Kelley Marie Kemper Haarberg. (2011).** Nutraceutical efficacy in experimental animal models of inflammatory bowel disease: *Echinacea angustifolia, Prunella vulgaris* and *Hypericum gentianoides*. Graduate Theses and Dissertations. Iowa State University. Paper 10379.
17. **Kosaraju J, Chinni S, Roy PD, Kannan E, Antony AS, Kumar MS. (2014).** Neuroprotective effect of *Tinospora cardifolia* ethanol extract on 6-hydroxy dopamine induced parkinsonism. Indian J Pharmacol 46:176-80.
18. **Kuroda M, Yoshida N, Ichikawa H, Takagi T, Okuda T, Naito Y**. **(2006).** Lansoprazole, a proton pump inhibitor, reduces the severity of indomethacin-induced rat enteritis. Int J Mol Med.17:89-93.
19. **Leite AZ, Sipahi AM, Damia C, Coelho M, Garcez AT, Machado MC**. **(2001).** Protective effect of metronidazole on uncoupling mitochondrial oxidative phosphorylation induced by NSAID: a new mechanism. Gut; 48:163-7
20. **Lim YJ, Chun HJ. (2013).** Recent advances in NSAIDs-induced enteropathy therapeutics: new options, new challenges. Gastroenterol Res Pract ; 2013:761060.
21. **Matsui H, Shimokawa O, Kaneko T, Nagano Y, Rai K, Hyodo I**. **(2011).** The pathophysiology of non steroidal anti-inflammatory drug (NSAID)-induced mucosal injuries in stomach and small intestine. Clin. Biochem. Nutr mar; 48(2):107-11.
22. **Magolis SA, Howell BF, Schaffer R. (1977).** Lactate dehydrogenase inhibitors in NADH preperations. Clin Chem; 23(9):1581-4.

23. **Molodecky NA, Soon IS, Rabi DM, Ghali WA, Ferris M, Chernoff G. (2012).** Increasing incidence and prevalence of the inflammatory bowel diseases with time, based on systemic review. Gastroenterol Rep (Oxf); 142:46-54.
24. **Mueller. D, Triebel. S, Rudakovski.O, Richling. E. (2013).** Influence of triterpenoids present in apple peel on inflammatory gene expression associated with inflammatory bowel disease (IBD). Food Chemistry; 139: 1–4, Pages 339-346.
25. **Neurath M. F, Finotto S, Fuss I, Boirivant M , Galle P . R & Strober W. (2001).** Regulation of T-cell apoptosis in inflammatory bowel disease: to die or not to die, that is the mucosal question. Trends. Immunol; 22: 21 – 26
26. **Ohkawa H, Ohishi N, Yagi K. (1979 Jun)**. Assay for lipid peroxides in animal tissues by thiobarbituric acid reaction. Anal Biochem; 95(2):351-8
27. **OECD guidelines for testing of chemicals (2001);** 423, Adopted: 17th December 2001
28. **Podolsky DK. (2003).** The future of IBD treatment. J Gastroenterol. 38:63–6.
29. **Podolsky , D . K . (1991).** Infl ammatory bowel disease . N. Engl. J. Med. 325, 928 – 937
30. **Podolsky , D . K . (2002).** Infl ammatory bowel disease. N. Engl. J. Med. 347, 417 – 429
31. **Sartor , R . B. (1994).** Cytokines in intestinal inflammation: pathophysiologic and clinical considerations. Gastroenterology. 106, 533 – 539
32. **Sarrazin S, Mossadegh-Keller N, Fukao T, Aziz A, Mourcin F, Vanhille L. (2009).** MafB restricts M-CSF-dependent myeloid commitment divisions of hematopoietic stem cells. Cell; 138:300–13
33. **Sartor RB. (1997).** How relevant to human inflammatory bowel disease are current animal models of intestinal inflammation. Aliment Pharmacol Ther; 11 (suppl.3): 89-97.
34. **Saha S, Ghosh S. (2012 Jun).** *Tinospora cardifolia* one plant, many roles. Anc Sci Life 31(4):151-9.
35. **Saitta KS, Zhang C, Lee KK, Fujimoto K, Redinbo MR. (2014 jan).** Bacterial β-glucuronidase inhibition protects mice against enteropathy induced by indomethacin, ketoprofen or diclofenac:mode of action and pharmacokinetics. Xenobiotica; 44(1):28-35.
36. **Sales H, Basso PJ. (2015).** Classical and recent advances in the treatment of inflammatory bowel disease. Braz J Med Biol Res; 48(2):96-107.
37. **Santucci. L, Agostini. M, Bruscoli .S, Mencarelli. A, Ronchetti .S, Ayroldi. E, Morelli. A, Baldoni. M, and Riccardi. C. (2007 Jan).** GITR modulates innate and adaptive mucosal immunity during the development of experimental colitis in mice. Gut; 56(1): 52–60.
38. **Seril DN, Liao J, Yang CS, Yang CS. (2003).** Oxidative stress and ulcerative colitis-associated carcinogenesis: studies in humans and animal models. Carcinogenesis. 24:353-62.
39. **Sinha K, Mishra NP, Singh J. (2004 jul).** *Tinospora cardifolia* (Guduchi), a reservoir plant for therapeutic applications. Indian J TradSci.3 (3):257-70.
40. **Spahn T W and Kucharzik, T. (2004 Mar).** Modulating the intestinal immune system: the role of lymphotoxin and GALT organs. Gut; 53(3): 456–465.
41. **Sudeesh NP, Ajith TA, Janardhanan KK. (2013).** Ganoderma lucidum ameliorate mitochondrial damage in isoproterenol-induced myocardial infarction in rats by enhancing the activities of TCA cycle enzymes and respiratory chain complexes. Int J Cardio; 165:117-25.
42. **Takagi T, Naito Y, Okada H, Takaoka M, Iyo TO, Yamada S. (2012).** Hemopexin is upregulated in rat intestinal mucosa injured by indomethacin. J Gastroenterol Hepatol Res; 27(3):70-5.
43. **Triantafillidis JK, Merikas E. (2011).** Current and emerging drugs for the treatment of inflammatory bowel disease. Drug Des DevelTher; 5:185-210.
44. **Wiess SJ, Sliva A. (1982 feb).** Monocyte and granulocyte-mediated tumor cell destruction, a role for the hydrogen peroxide-myeloperoxidase chloride system. J Clin Invest; 69:255-62.
45. **Yamamoto A, Itoh T, Nasu R, Nishida R. (2014 mar).** Sodium alginate ameliorates indomethacin-induced gastrointestinal mucosal injury via inhibiting translocation in rats. World J Gastroenterol. 14; 20(10):2641-52.
46. **Yanhua X, Chen X, Wang W. (2011).** The progress of mesenchymal stem cells repaired inflammatory bowel disease. Chongqing Med; 40:293–6.
47. **Yokota A, Taniguchi Y, Tanaka A, Takeuchi K. (2005 apr).** Rofecoxib produces intestinal but gastric damage in the presence of a low dose of indomethacin in rats. J Pharmacol Exp Ther. 4; 314:302-309.