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AMELIORATION OF DEXTRAN SULPHATE SODIUM INDUCED ULCERATIVE COLITIS IN MICE BY STEM BARK EXTRACT OF *TERMINALIA TOMENTOSA*

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Terminalia tomentosa, IBD, Ulcerative colitis, Oxidative stress

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ABSTRACT: A plethora of herbs have been tested for their activity against ulcerative colitis, a debilitating disease in the group of IBDs. However, a promising adjuvant to conventional therapy remains still at large. Ellagic acid, a polyphenol widely available in berries have shown efficacy against ulcerative colitis in preclinical models. Owing to its high content of ellagic acid and its analogs, the stem bark of *Terminalia tomentosa* was chosen for our study. Effect of *Terminalia tomentosa* hydroalcoholic extract and its ellagic acid-rich fraction were tested for their anti-colitic potential in DSS (5%) induced ulcerative colitis in mice. The evaluation was carried out for assessing the severity of disease at macroscopic biochemical and histopathological level. Myeloperoxidase and superoxide anions, an inflammatory and an oxidative marker respectively were also evaluated in all the groups. Amongst all, the highest significance was observed in the group treated with an ellagic acid-rich fraction (200 mg/kg). The ameliorative effect has been attributed to the high antioxidant potential of the fraction.

INTRODUCTION: Inflammatory bowel diseases (IBD) such as Crohn's Disease and Ulcerative Colitis have a high propensity of occurrence globally. Among the two, ulcerative colitis, an idiopathic chronic ulcero inflammatory condition, is more epidemiologically relevant and is extending it's at a faster pace in the modern world [1].

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Changing food habits and autoimmune anomalies both have their stakes in the aetiopathogenesis of the disease, however in spite of pinning down the pathological basis since a long time we have failed to hit upon an effective strategy which can address the various facets of its complex pathology [2].

Current therapeutic approaches rely mainly on anti-inflammatory agents like sulphasalazine or corticosteroids in combination with immunosuppressants or monoclonal antibodies. These agents though effective to some extent often fail to show clinical benefits on a chronic course hence necessitating the lookout for an effective therapeutic agent which still seems to be at large.

The stem bark of *Terminalia tomentosa* (Roxb.), commonly known as crocodile bark tree, belonging to the family *Combretaceae* though rich in various phytoconstituents of medicinal importance, has largely remained off notice through the years. However, the plant has gained focus in the recent past owing to the works of some zealous groups which have characterized the spectrum of medicinal potential of its various constituents. Predominant among those are anti-inflammatory, antimicrobial and antidiarrhoeal activities [3, 4]. The plethora of medicinal properties of the plant has been attributed largely to its rich phenolic content especially in the stem bark. The stem bark of *Terminalia tomentosa* has been reported to be a trove of medicinal components among which ellagitannins like ellagic acid and its analogs are predominant [5, 6]. Ellagitannins from a variety of herbs have shown promising therapeutic effects against ulcerative colitis and other gastrointestinal pathologies [7]. Anti-colitic efficacy of ellagic acid microspheres has also been documented earlier [8]. However, to the best of our knowledge, the anticolitic potential of a plant-like *Terminalia tomentosa* which is reported to be high in ellagic acid content has not been explored as yet. Hence, we found a good rationale behind carrying out a comparative evaluation of the hydroalcoholic extract of *Terminalia tomentosa* stem bark (TTE) and its ellagic acid-rich fraction (TTEA) against colitic flare which to the best of our knowledge is a pioneering effort of its kind.

MATERIALS AND METHODS:

Chemicals: Dextran sulfate sodium salt (Mol. wt. 36000 - 50,000) from Sumona Enterprise, Bangalore, Nitroblue tetrazolium from Loba Chem, Mumbai. o-Dianisidine hydrochloride was procured from S.D Fine Chemicals Ltd., Mumbai. The chemicals were all of analytical grade.

Plant Material: The stem bark of the plant *Terminalia tomentosa* (Roxb.) were collected from the Barjora Range of Bankura, West Bengal and after due identification by competent authority a voucher specimen was deposited at Central National Herbarium, BSI, Shibpur (Voucher no. : CNH/43/2018/Tech.II/69)

Animal Husbandry & Statutory Approval: Treatment naïve Swiss albino mice of weight 22 - 25 g and of age 6-8 weeks were chosen for the study. Animals were harbored under standard housing conditions as prescribed in the guidelines of CPCSEA, India for maintenance and care of laboratory animals. Briefly, a 12 h light /dark cycle with a constant temperature of 22 °C - 25 °C and access to food and water *ad libitum*. Prior permission, to carry out the procedures proposed in the protocol (Ref. no. GCTS/IAEC/2018-Sep/04), was duly obtained from the Institutional Animal Ethics Committee (IAEC) of GCTS, Asansol (Regd no. 955/A/06/CPCSEA)

Preparation of *Terminalia Tomentosa* Stem Bark Hydroalcoholic Extract: The inner layers of the bark of the plant (*T. tomentosa*) were removed and washed gently with fresh water to remove the residual dust particles. The bark was shade dried and minced to small pieces. Then the small pieces were grinded by using a Willey grinder to form a powder and sifted through sieve number 60. The dry powdered stem bark of *Terminalia tomentosa* (200 g) was soaked in about 1000 ml ethanol-water mixture (3:1) for seventy-two hours at room temperature with occasional shaking intermittently. The macerate was then sieved through a clean muslin cloth and was filtered by a filter paper. The solvent was then evaporated by rotary vacuum evaporator for producing solvent free extract. Finally, about 3 g of methanolic extract was obtained. The extract was stored in an airtight container at 4 °C until further use.

Preparation of Ellagic Acid Rich Fraction of *Terminalia Tomentosa*: The fraction of the stem bark extract of *Terminalia tomentosa*, rich in ellagic acid was prepared by the method described previously by Singh *et al.*, in 20092 with slight modification. Briefly, the dried stem bark powder of *Terminalia tomentosa* was extracted at 60 °C - 70 °C by using aqueous ethanol (50%) as the solvent. Subsequently, ethanol was removed from the mixture under reduced pressure. The aqueous portion left was treated with hydrochloric acid and refluxed at 70 °C for 6 h. This was diluted with water and upon dilution ellagic acid was precipitated. The precipitate was collected by filtration and dried in vacuum oven.

Induction of Ulcerative Colitis: For induction of ulcerative colitis drinking water was supplemented

with DSS (5% w/v solution) for a period of seven days. The choice of the induction period was dictated by the exhibition of ceiling effects in pathological signs by the animals. The water intake and the condition of the general health of the animals in all groups were kept under strict vigil so as to ensure even exposure to the inducer and onset of symptoms respectively.

Disease Activity Index: Onset of disease was assessed by estimating the disease activity index by the method described previously by Maheshwari *et al.*, in 2015 [9] with necessary modifications. The scoring system deployed for assessment has been depicted below:

TABLE 1: SCHEME FOR SCORING DISEASE ACTIVITY INDEX

Score	Weight loss (%)	Stool consistency	Fecal occult blood test or gross bleeding
0	0	Soft pallets	Normal
1	1-10	Semi solid	Guaiac (+)
2	11 -15	Pasty stool	Guaiac (+ +)
3	16 -20	Loose stool	Gross bleeding
4	>20	Watery Stool	Gross bleeding

Is ease activity index was calculated by the following formula as proposed by Singh *et al*.: [2]

DAI = Weight loss + Stool Consistency + FOB/GB/3

Macroscopic Assessment of Ulcerative Damage: The transacted colon was sectioned longitudinally to unfurl the luminal mucosa for examination by a blind examiner. The criteria described previously by Gupta *et al.*, in 2015 [10] was used for grading the extent of damage based on some predefined parameters **Table 2**.

TABLE 2: SCHEME FOR SCORING MACROSCOPIC DAMAGE

Parameters	Score
No macroscopic changes	0
Isolated mucosa erythema	1
Mild mucosal erythema, minimal bleeding & erosion	2
Moderate erythema, bleeding ulcers & small erosions	3
Severe ulceration, bleeding & erosion with tissue necrosis	4

Determination of Colonic Length and Weight Ratio: Colonic length and weight are important indicators of organ-specific damage as far as ulcerative colitis is concerned. Hence, a good rationale is found in assessing these parameters to decipher the extent of damage in the colons of the animals under investigation. The process is a modification of the one previously described by Dugani *et al.*, in 2016 [11]. Briefly, the animals were euthanized by standard protocol and the colons were excised from the caecoanal junction up to the pelvis renal eminence. The colonic length was measured as a parameter of atrophy and tissue wasting resulting from colitic damage. The relative proportion of the colonic length and weight was thus measured as a marker for pathological damage.

Determination of Myeloperoxidase Activity: Myeloperoxidase is an enzyme released from the neutrophils accumulating in the region of inflammation and hence may serve as an indicator of the severity of inflammatory damage. Myeloperoxidase in tissue was estimated by the method described previously by Salaga *et al.*, in 2017 [12] with slight modification. Briefly, a small segment of colonic tissue was weighed, chopped and subsequently homogenized in ten volumes of ice-cold potassium phosphate buffer of pH 7.4. The homogenate was subjected to centrifugation at 3500 rpm for 30 min at 4 °C. Next the pellet was suspended in 10 ml of ice-cold potassium phosphate buffer (50 mm, pH 6) which contained hexadecyltrimethylammonium bromide (HTAB) in 10mM EDTA. The resuspended pellet was put into an alternate freeze-thaw cycle followed by a brief period of sonication (15 sec). The solution was once more centrifuged at 15000 rpm for 20 min at 4 °C and spectrophotometric determination of myeloperoxidase activity was done at 460 nm.

Determination of Lipid Peroxidation: Malondialdehyde (MDA) was estimated in colon as an index of lipid peroxidation to determine the extent of oxidative injury. The process was a slight modification of the one described previously by 13. Briefly, the supernatant fraction (2 ml) of 10% tissue homogenate was mixed with an equal volume of 0.67% (w/v) thiobarbituric acid and placed in a boiling water bath for 10 min. The sample was cooled by placing in an ice bath for 5 min and then the absorbance was read at 532 nm wavelength in UV-VIS Spectrophotometer (Spectrascan UV 2600, Chemito). The concentration of malondialdehyde (MDA) in the

tissue was then calculated from the standard curve and expressed as micromole (µM) of malondi-aldehyde mg^{-1} wet tissue.

Determination of Superoxide Dismutase Activity: The superoxide dismutase activity in the colon was determined by using the Superoxide dismutase kit from R&D Systems Inc. (Cat No. 7500-100-K) as per the manufacturer's protocol. Superoxide dismutase activity was gauged in terms of pmol of reduced NBT/min/mg of tissue.

Histopathological Examination: The colons were fixed in 10% formalin and 5 µm sections were subjected to routine staining by hematoxylin and eosin for determining the extent of damage in the colon. The severity of damage was evaluated by deploying the system of scoring proposed originally by Cosin *et al.*, in 2015 [14] with slight modification. The scheme is depicted below.

TABLE 3: SCHEME FOR HISTOLOGICAL SCORING. PMNC: POLYMORPHONUCLEAR CELLS

Features	Score
Normal histological appearance	0
Mild damage extending up to epithelial surface	1
Mild to moderate damage with PMNC infiltration	2
Moderate damage with crypt destruction	3
Widespread crypt destruction enclosed by normal tissue	4
Multiple ulcerated foci enclosed by grossly Distorted tissue texture	5

Experimental Design: Seven groups (n = 6/group) were deployed for the study, the details of which are furnished below:

Group I (Vehicle Control): Mice were treated once daily with vehicle (0.5% carboxymethyl cellulose) for 7 days. On day 8 upon sacrifice, the colonic tissues were collected and subjected to macroscopic, biochemical and histopathological examination.

Group II (DSS Control): Mice were supplied 5% DSS as a supplement to drinking water for 7 days. On day 8 upon the sacrifice, the colonic tissues were collected and subjected to macroscopic, biochemical and histopathological examination.

Group III (Treated with Hydroalcoholic Extract of *Terminalia tomentosa* 100 mg/kg): Mice were treated once daily with 100 mg/kg of *Terminalia tomentosa* ethanolic extract (TTE 100 mg/kg) starting 3 days prior to the induction and spanning thereafter for 7 consecutive days post-induction. On day 8 post-induction, mice were sacrificed and the colonic tissues were collected for subjecting those to macroscopic, biochemical and histo-pathological examination.

Group IV (Treated with Hydroalcoholic Extract of *Terminalia tomentosa* 200 mg/kg): Mice were treated once daily with 100 mg/kg of *Terminalia tomentosa* ethanolic extract (TTE 200 mg/kg) starting 3 days prior to the induction and spanning thereafter for 7 consecutive days post-induction. On day 8 post-induction, mice were sacrificed and the colonic tissues were collected for subjecting those to macroscopic, biochemical and histopathological examination.

Group V (Treated with Ellagic acid-rich fraction of Ethanolic Extract of *Terminalia tomentosa* 100 mg/kg): Mice were treated once daily with 100 mg/kg of Ellagic acid-rich fraction of *Terminalia tomentosa* stem bark (TTEA 100 mg/kg) starting 3 days prior to the induction and spanning thereafter for 7 consecutive days post-induction. On day 8 post-induction, mice were sacrificed and the colonic tissues were collected for subjecting those to macroscopic, biochemical and histopathological examination.

Group VI (Treated with Ellagic acid-rich fraction of Ethanolic Extract of *Terminalia tomentosa* 200 mg/kg): Mice were treated once daily with 100 mg/kg of Ellagic acid-rich fraction of *Terminalia tomentosa* stem bark (TTEA 200 mg/kg) starting 3 days prior to the induction and spanning thereafter for 7 consecutive days post-induction. On day 8 post-induction, mice were sacrificed and the colonic tissues were collected for subjecting those to macroscopic, biochemical and histopathological examination.

Group VII (Treated with Sulphasalazine (100 mg/kg): Mice were treated once daily with 100 mg/kg of Sulphasalazine, starting 3 days prior to the induction and spanning thereafter for 7 consecutive days post-induction. On day 8 post-induction, mice were sacrificed and the colonic tissues were collected for subjecting those to macroscopic, biochemical and histopathological examination.

Statistical Analysis: The results were expressed as mean ± SEM. One way ANOVA was used to analyze the significance of data obtained from different groups statistically except for the DAI scores which were analyzed by using two ways ANOVA. Tukey's multiple range tests and Bonferroni's test were used for post hoc analysis of the results obtained from one way ANOVA and two ways ANOVA respectively

RESULTS:

Attenuation of DSS Induced Disease Severity by *Terminalia Tomentosa*: DSS treatment showed a progressive increase in disease severity as reflected by the ascending trend of the disease activity index score in the animals of the DSS treated group and the same showed the ceiling effect consecutively on the fifth day and seventh day of dosing. The disease severity, however, got declined in the animals treated with hydroalcoholic extract of *Terminalia tomentosa* (200 mg/kg) or its ellagic acid-rich fractions (100 mg/kg & 200 mg/kg) as depicted in the graph **Fig. 1** though greater significance was observed only in the fraction treated groups.

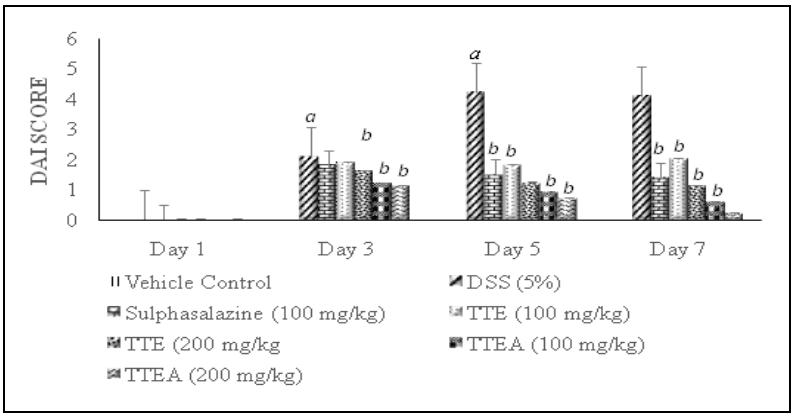

FIG. 1: EFFECT OF DSS INDUCED COLITIS ON DISEASE ACTIVITY INDEX SCORES IN DIFFERENT GROUPS OF MICE DUE TO TREATMENT WITH HYDROALCOHOLIC EXTRACT OF *TERMINALIA TOMENTOSA*, ITS ELLAGIC ACID RICH FRACTION AND STANDARD DRUG SUPHASALAZINE (100 mg/kg) Results expressed as mean ± SEM. a p<0.05 comparison to the vehicle control group; b p<0.05 in comparison to DSS control group

TABLE 4: COLONIC LENGTH AND WEIGHT RATIO VALUES EXPRESSED AS ± SEM (P<0.05)

Groups	Treatment	Colonic Length/Weight Ratio
1	Vehicle Control	4.6 ± 0.01
2	DSS (5%)	0.6 ± 0.06^{x}
3	TTE (100 mg/kg)	0.92 ± 0.04
4	TTE (200 mg/kg)	2.4 ± 0.04^{y}
5	TTEA (100 mg/kg)	3.2 ± 0.04^{y}
6	TTEA (200 mg/kg)	4.3 ± 0.05^{y}
7	Sulphasalazine	3.9 ± 0.02^{y}

x p<0.05 in comparison to the vehicle control group; y p<0.05 in comparison to DSS control group

Effect of *Terminalia tomentosa* on DSS Induced Alteration in Colonic Length and Weight Ratio: Successful induction of colitis by dextran sulfate sodium (5%) was reflected by the significant reduction of colonic length and weight ratio in the colitic control group as compared to that of the animals in the vehicle control group **Table 4**. The evidence of ameliorative potential of *Terminalia tomentosa* was, on the other hand, proved by preservation of the ratio in the group treated with *Terminalia tomentosa* hydro alcoholic extract or its ellagic acid-rich fractions though the effects were significant only in the fraction treated groups which were comparable to that of the standards.

Alteration of Macroscopic Indices of Colitic Pathology and Histopathological Scores in DSS Treated Animals by *Terminalia tomentosa*: Macroscopic changes in the colon were widespread in animals belonging to DSS treated group. The changes were gauged in terms of edema, erythema in mucosa, erosion, ulceration and goblet cell depletion. Histopathological evaluation was also performed with colonic sections of thickness 5 µm. on the basis of epithelial distortion, ulceration and extent of inflammatory infiltrate. All of these pathological artifacts were however significantly attenuated in the animals treated with hydroalcoholic extract of *Terminalia tomentosa* (100 mg/kg & 200 mg/kg) or its ellagic acid-rich fractions (100 mg/kg & 200 mg/kg) as depicted in the graph **Fig. 2**, **Table 5** though the effects were significant only in the fraction treated groups.

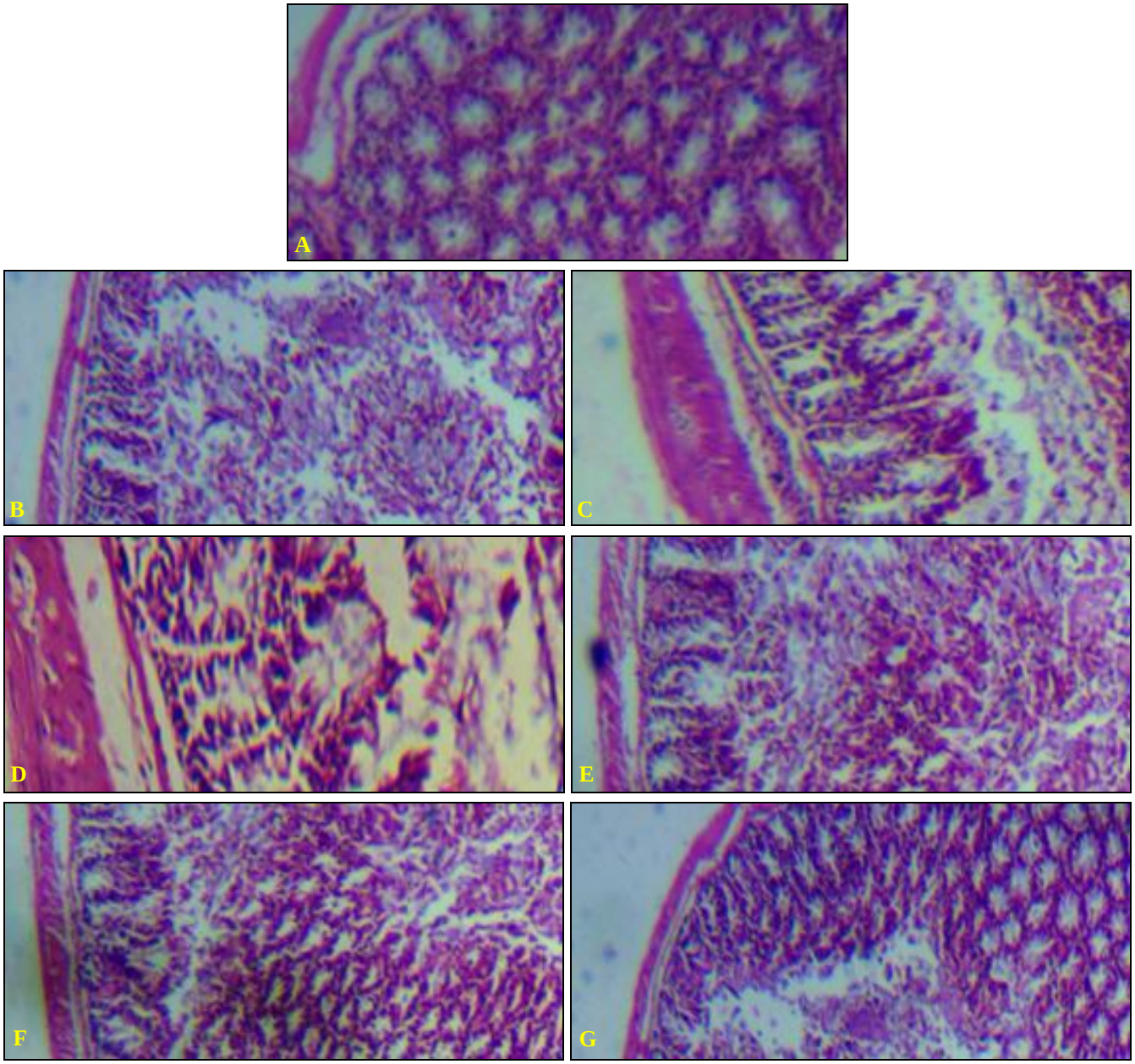

FIG. 2: AMELIORATION OF HISTOPATHOLOGICAL CHANGES INDUCED BY DSS ON COLONIC TISSUE. All snaps have been taken at 20X magnification. (A) Section showing physiological texture with preservation of crypts (vehicle control). (B) Section showing widespread destruction of crypts along with robust changes in tissue architecture at the epithelial and submucosal level (DSS control). (C) Relative restoration of cryptic texture and villous structures with the standard drug (Sulphasalazine 100 mg/kg). [D TTE (100 mg/kg) & E (200 mg/kg)] Initiation of cryptic restoration and reappearance of tissue architecture in the TTE (200 mg/kg) group. [F TTEA (100 mg/kg) & G (200 mg/kg)] Establishment of restored cryptic texture reconstitution of the tissue architecture at epithelial level in TTEA (200 mg/kg) treated group.

TABLE 5: EFFECT OF DSS INDUCED COLITIS ON HISTOPATHOLOGICAL AND MACROSCOPIC SCORE IN DIFFERENT GROUPS OF MICE DUE TO TREATMENT WITH HYDROALCOHOLIC EXTRACT OF *TERMINALIA TOMENTOSA*, ITS ELLAGIC ACID RICH FRACTION AND STANDARD DRUG SUPHASALAZINE (100 mg/kg)

Group	Treatment	Mac score	Histo score
1	Vehicle control	0 ± 0	0 ± 0
2	DSS (5%)	3.8 ± 0.3^{x}	4.0 ± 0.5^{x}
3	TTE (100 mg/kg)	2.9 ± 0.4	2.5 ± 0.6
4	TTE (200 mg/kg)	1.8 ± 0.6^{y}	1.4 ± 0.8^{y}
5	TTEA (100 mg/kg)	1.0 ± 0.3^{y}	1.1 ± 0.06^{y}
6	TTEA (200 mg/kg)	0.5 ± 0.4^{y}	0.4 ± 0.02^{y}
7	Sulphasalazine	0.6 ± 0.6^{y}	0.6 ± 0.3^{y}

Results expressed as mean ± SEM. ^{x} $p<0.05$ in comparison to vehicle control group; ^{y} $p<0.05$ in comparison to DSS control group

Attenuation of DSS Induced Rise in Myeloperoxidase Activity by *Terminalia tomentosa*: Myeloperoxidase activity was found to be significantly hiked in DSS induced colitic animals in comparison to those of the vehicle-treated group. The same however got declined in the animals treated with hydroalcoholic extract of *Terminalia tomentosa* (100 mg/kg & 200 mg/kg) or its ellagic acid-rich fractions (100 mg/kg & 200 mg/kg) as depicted in the graph **Fig. 3** though significance in comparison to the colitic group was observed only in the fraction treated animals.

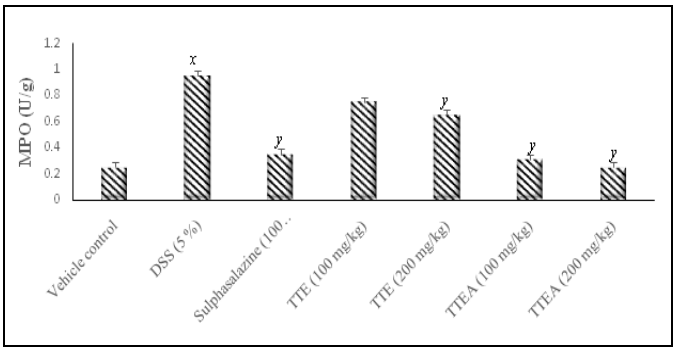

FIG. 3: EFFECT OF DSS INDUCED COLITIS ON LEVELS OF MPO IN DIFFERENT GROUPS OF MICE DUE TO TREATMENT WITH HYDROALCOHOLIC EXTRACT OF *TERMINALIA TOMENTOSA*, ITS ELLAGIC ACID RICH FRACTION AND STANDARD DRUG SUPHASALAZINE (100 mg/kg) Results expressed as mean ± SEM. x $p<0.05$ in comparison to the vehicle control group; y $p<0.05$ in comparison to DSS control group

Attenuation of DSS Induced Oxidative Stress by *Terminalia tomentosa*: Significant hike in oxidative markers like thiobarbituric acid reactive substances (TBARS) and superoxide anions were observed in the animals of the group treated with DSS. On the other hand, the aforementioned oxidative markers registered a sharp decline in the groups treated with hydroalcoholic extract of *Terminalia tomentosa* (100 mg/kg & 200 mg/kg) or its ellagic acid-rich fractions (100 mg/kg & 200 mg/kg) as depicted in the graph **Fig. 4** & **Fig. 5** though significance in comparison to the colitic group was observed only in the fraction treated animals.

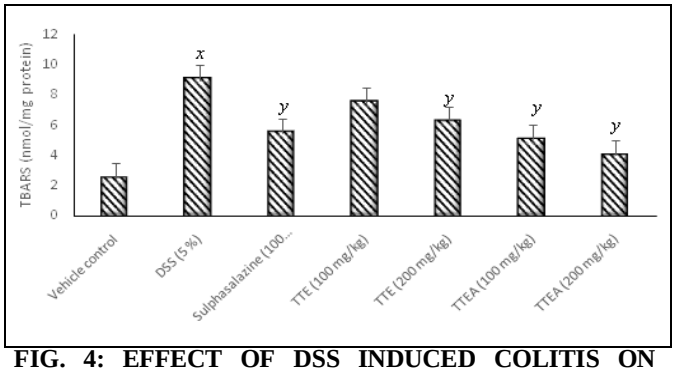

FIG. 4: EFFECT OF DSS INDUCED COLITIS ON LEVELS OF MALONALDEHYDE IN DIFFERENT GROUPS OF MICE DUE TO TREATMENT WITH HYDRO ALCOHOLIC EXTRACT OF *TERMINALIA TOMENTOSA*, ITS ELLAGIC ACID RICH FRACTION AND STANDARD DRUG SUPHASALAZINE (100 mg/kg) Results expressed as mean ± SEM. x $p<0.05$ in comparison to vehicle control group; y $p<0.05$ in comparison to DSS control group

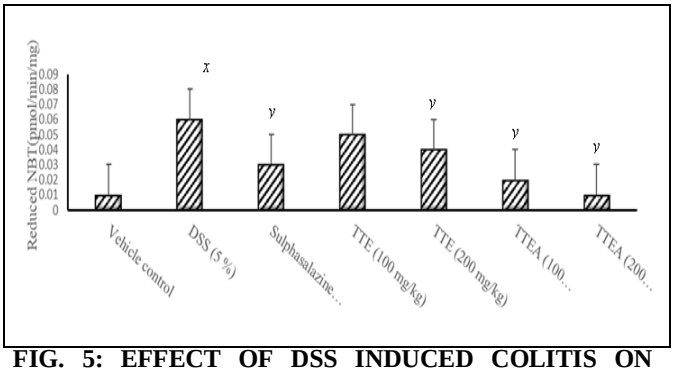

FIG. 5: EFFECT OF DSS INDUCED COLITIS ON LEVELS OF REDUCED NBT IN DIFFERENT GROUPS OF MICE DUE TO TREATMENT WITH HYDRO ALCOHOLIC EXTRACT OF *TERMINALIA TOMENTOSA*, ITS ELLAGIC ACID RICH FRACTION AND STANDARD DRUG SUPHASALAZINE (100 mg/kg) Results expressed as mean ± SEM. x $p<0.05$ in comparison to vehicle control group; v $p<0.05$ in comparison to DSS control group

DISCUSSION: The spectrum of symptoms manifested by the patients having an acute or chronic course of IBD can be elicited aptly in animals either in a lymphocyte dependent or lymphocyte independent manner [15]. The former one, though holds the promise for greater homology, strain-specific differences in immune responses restrict its rate of success [12]. Dextran sulfate sodium (DSS) induced colitis, primarily a lymphocyte independent model, has thus evolved as the most preferred model reflecting successfully the majority of the pathological features prevalent

in IBD patients especially those suffering from ulcerative colitis. Macroscopic features like mucosal erythema, bleeding, epithelial erosion and focal ulceration.

Commonly observed in DSS induced murine colitis [16] though showed signs of decrease in the groups treated by both doses of hydroalcoholic extract of stem bark of *Terminalia tomentosa* (100 mg/kg and 200 mg/kg) in comparison to positive control group the achievement of statistically significant reduction only with the two doses of the ellagic acid fractions quite similar to that obtained with the standard group (Sulphasalazine 100 mg/kg) indicates the predominant role of ellagic acid in the beneficial role of the plant against ulcerative colitis. The idea is further strengthened by obtaining similar kind of results with the histopathological evaluation. The anti-colitic potential of ellagic acid-rich fractions also finds support in the works reported earlier by various groups [17] who made successful use of ellagic acid microspheres in treating DSS induced colitis in preclinical samples.

Terminalia tomentosa (Roxb.) has been used in folklore medicine as a wound healing, anti-inflammatory and anti-diarrhoeal agent [18] though reports on the scientific validation of the plant's medicinal potential are still scanty with noteworthy contributions from only a handful of workers. The traditional use of *Terminalia tomentosa* in GI disorders together with the documented predominance of ellagic acid and its analogs in the stem bark extract of the plant [19] prompted us to carry out a comparative study between its ethanolic extract and ellagic acid-rich fraction with regard to their potential against ulcerative colitis. Convincing evidence of the ellagic acid-rich fractions' ameliorative capacity could be found in the differential cumulative Disease Activity Indices of the animals across the groups.

Statistical analysis of our results revealed that the attenuation of DSS induced colitis pathology were of greater significance in the fraction treated groups with the effect being most prominent in the group treated with 200 mg/kg of ellagic acid-rich fraction. Colonic length and weight ratio which serves as an important index in evaluation of disease onset also showed the most significant hike in the fraction (200 mg/kg) treated groups which were comparable to the groups treated with sulphasalazine (100 mg/kg).

The aforementioned evidences paved the way for further probing into the other parameters reportedly associated with colitis. Heavy neutrophilic infiltration with a concomitant rise in secretion of its oxidative enzyme myeloperoxidase (MPO) is a recognized feature of ulcerative colitis [20] and contributes largely to the aggravation of its inflammatory pathology [21]. In our study the colons of the DSS induced positive control group was found to creep on the documented line and showed a significant rise in MPO levels compared to the vehicle control group and amongst the test groups the fraction (200 mg/kg) treated group showed the most significant drop compared to the positive control akin to that of the standard ones. Thus, it may be the case that Ellagic acid, a documented anti-inflammatory agent [21] may be the main player behind the anticolitic effect of *Terminalia tomentosa*.

In the continuum of the above it can be very well deciphered that the free radical injury inflicted by the accumulating neutrophils in the zone of inflammation may contribute significantly in the aggravation of pathology of the disease which makes it inevitable to check the oxidative markers as an accessory parameter of the pathological status.

In our study we evaluated two signature markers of oxidative stress namely MDA and superoxide anions. The former one was gauged by the quantum of rise in TBARS in the colon and the latter by the quantum of reduced NBT in the colonic tissue. Both the oxidative markers showed significant decrease in the fraction treated groups however the most significant decrease was registered in the animals treated with 200 mg/kg of the fraction.

CONCLUSION: Hydroalcoholic extract of stem bark of *Terminalia tomentosa*, especially its ellagic acid-rich fraction can be instrumental in amelioration of DSS induced ulcerative colitis, and the activity may largely be attributed to its antioxidant potential.

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CONFLICTS OF INTEREST: There are no conflicts of interest

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