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Pharmacological Effect of the *H. indicus* Herbal Formulation on Experimental Models for Inflammatory Bowel Disease

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KEYWORDS

Hemidesmus indicus Linn , Inflammatory Bowel Disease (IBD), Crohn's disease, ulcerative colitis.

ABSTRACT:
The medicinal plants possessing natural antioxidant and anti-inflammatory properties are used since ancient times for curing several healthcare issues. The present study was done for evaluation of Pharmacological effect of the H. indicus herbal formulation on experimental models for Inflammatory Bowel Disease(IBD). The models used for study of IBD are acetic acid induced ulcerative colitis and indomethacin induced enterocolitis. Intrarectal instillation of acetic acid in rats affected only the distal colon portion. These Phytochemical compounds of Hemidesmus indicus root bark have been found to induce a wide spectrum of activities such as reduction in oxidative stress, suppression of inflammation, and cell proliferation and modulating numerous signal transduction pathways. Acetic acid raised the levels of colonic MPO, indicating infiltration of neutrophils and perturbation of the inflammatory system. This fact is documented in animal models, with IBD. H. indicus root bark Linn. bark extract ameliorated neutrophil infiltration as evidenced by suppression of colon MPO and improvement of histological features. Histopathology examination of drug treated group revealed less damage compared to control group. A significant decrease in MPO activity was also observed. All these observations support the findings that the barks extract of H. indicus root barkLinn. was able to offer significant protection in both the models studied. Acetic acid-induced colitis and indomethacin-induced enterocolitis simulate two different disease conditions, which are ulcerative colitis and Crohn's disease respectively. On this basis we can say that the constituents extract under study may be useful in treating UC as well as CD in humans. The prednisolone treatment has shown significant protection in both the animal models under our study. The bark extract H. indicus root barkLinn. was found comparable with standard drug. The role of oxygen-derived free radicals, such as hydroxyl radical and superoxide radical, in the inflammatory process is well known. It is also generally assumed that most of the antioxidants possess anti-inflammatory effect. Bark extract of H. indicus root barkLinn. may account for the observed anti-inflammatory properties.

INTRODUCTION

Hemidesmus indicus Linn. R. Br. (Fam. Asclepidiaceae) is a prostrate or semi-erect shrub found throughout India from upper Gangetic plains east-wards to Assam, throughout Central, Western and Southern India upto an elevation of 600m. Hemidesmus indicus Linn. belongs to a family Asclepidaceae is commonly found throughout all parts of India. It is known as Ananta and Sariva in Sanskrit, Upalsari in Gujarati, Anantamula in Hindi and Indian sarsaparilla in English. It is widely used as tonic, demulcent, diaphoretic,

Journal of Chemical Health Risks

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diuretic and blood purifier[1]. The plant is being used against syphilis, leucorrhoea, bronchitis, chronic rheumatism, urinary diseases, leprosy, leucoderma and skin diseases, and as purgative, diaphoretic, diuretic, antipyretic and antidiarrheal in folk medicines (3). The plant is used against diseases of blood, inflammation, diarrhea, respiratory disorders, skin diseases, syphilis, fever, bronchitis, asthma[2,3].

Inflammation plays a fundamental role in host defenses and the progression of immune-mediated disease[4]. The inflammatory response is initiated in response to injury (e.g. trauma, ischemia, and foreign particles) and infection (e.g. bacterial or viral infection) by multiple events, including chemical mediators (e.g. cytokines and prostaglandins) and inflammatory cells (e.g. leukocytes). It is characterized by increased blood flow to the tissue, causing pyrexia, erythema, induration and pain[5].

Inflammatory bowel disease (IBD) is an idiopathic chronic inflammatory condition of the gastrointestinal tract, comprising Crohn's disease and ulcerative colitis. The symptoms of IBD are chronic diarrhea, abdominal pain, rectal bleeding, weight loss, and shortening of the colon. Although the etiology of IBD remains uncertain, it is known that irregular immune response, gut microbial flora, and genetic and environmental factors are associated with the pathogenesis of IBD [6]. The present treatment regimen, including aminosalicylates, corticosteroids, biologics, and immunosuppressants, has therapeutic limits and leads to side effects [7]. Furthermore, recent failures of drug targets in IBD, such as IL-17, IL-13, interferon (IFN)-γ, and chemokine receptor (CCR)-9, have indicated that single target therapy for IBD is difficult owing to pathogenic heterogeneity [8]. Therefore, the discovery of alternative treatment options with multiple therapeutic targets is required. We expect that natural product formulations, such as medicinal plant extracts or traditional medicines, would offer excellent alternative therapies for IBD.

In recent decades, medicinal plant extracts, traditional medicines and their active components have been investigated for the development of novel anti-inflammatory drugs. Many patients with IBD are interested in alternative treatments because they are dissatisfied with the current conventional treatment [9]. However, no successful therapeutics for IBD based on natural products have been developed. Thus, we aimed to explore potent herbal formulations for the treatment of IBD by using a large-scale screening test.

MATERIAL AND METHOD

Collection and air drying of plant material, the root of H. indicus plant was taken from the Satpura region of Madhya Pradesh, India. It was verified by the botanologist Safia College Bhopal Madhya Pradesh under voucher specimen number of 147 Herbarium. A sample specimen of collected material was stored in the herbarium for future reference. The H. indicus root parts were dried under shade at 30 ^{0}C for 20 days.

Preliminary phytochemical analysis, TLC finger printing of methanolic extract of root bark of *H. indicus*[10]

The methanolic extract was subjected to preliminary phytochemical testing for the detection of major chemical groups (Table 1). The details of the tests are as follows:

Phenols test: The methanolic extract was spotted on a filter paper. A drop of phoshomolybdic acid reagent was added to the spot and was exposed to ammonia vapours (Blue coloration of the spot indicate the presence of phenols).

Braemer's test for tannins: To a 2–3 ml of methanolic extract, 10% alcoholic ferric chloride solution was added. (Dark blue or greenish grey coloration of the solution indicate the presence of tannins in the drug).

Liebermann-Burchardt test for steroids and terpenoids: To 1 ml of methanolic extract of drug, 1 ml of chloroform, 2–3 ml of acetic anhydride and 1 to 2 drops of concentrated sulfuric acid were added. (Dark green coloration of the solution indicate the presence of steroids and dark pink or red coloration of the solution indicate the presence of terpenoids).

Alkaloids: Adrop of methanolic extract was spotted on a small piece of precoated TLC plate and the plate was sprayed with modified Dragendorff's reagent. (Orange coloration of the spot indicate the presence of alkaloids).

Bornträger's test for anthraquinones: About 50 mg of methanolic extract was heated with 10% ferric chloride solution and 1 ml of concentrated hydrochloric acid. The extract was cooled, filtered and the filtrate

Journal of Chemical Health Risks

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JCHR (2025) 15(6), 3105-3118 | ISSN:2251-6727

was shaken with diethyl ether. The ether extract was further extracted with strong ammonia. (Pink or deep red coloration of aqueous layer indicate the presence of anthraquinones).

Shinoda test for flavonoids: To 2–3 ml of methanolic extract, a piece of magnesium ribbon and 1 ml of concentrated hydrochloric acid were added. (Pink red or red coloration of the solution indicate the presence of flavonoids in the drug).

The total phenolic content of the extract was estimated according to the method described by Singleton and Rossi. In brief the method is as follows: Stock solution (1 mg/ml) of the extract was prepared in methanol. From the stock solution, suitable quantity of the extract was taken into a 25 ml volumetric flask and 10 ml of water and 1.5 ml of Folin Ciocalteu reagent were added to it. The mixture was kept for 5 min, and then 4 ml of 20% sodium carbonate solution was added and made up to 25 ml with double distilled water. The mixture was kept for 30 min and absorbance of blue color developed was recorded at 765 nm. Percentage of total phenolics was calculated from calibration curve of gallic acid plotted by using the above procedure and total phenolics were expressed as % gallic acid.

Table 1: Preliminary Phytochemical screening of methanolic extract of *H. indicus* root bark.

S. No.	Taste	Presence/Absence	Test Perform
1	Phenols	+++	Phosphomolbdic acid test
2	Tannins	+++	Braemaers Test
3	Steroids	++	Libermann Burchardt Test
4	Terpenoids	+	Burchardt test
5	Alkaloids	-	Dragendrorff's Test
6	Anthraquinones	-	Borntrager test
7	Flavonoids	++	Shinodas test

– absent; + Traces; +++ Abundant

TLC finger print profile was established for methanolic extract using HPTLC. A stock solution (1 mg/ml) of the methanolic extract was prepared in methanol. Suitably diluted stock solution was spotted on pre-coated Silica gel G60 F254 TLC plates using CAMAG Linomat IV Automatic Sample Spotter and the plates were developed in solvent systems of different polarities to resolve polar and non-polar components of the extract[11]. The plates were dried at room temperature and scanned using TLC Scanner 3 (CAMAG) at 254 nm (absorbance/reflectance mode) and 366 nm (fluorescence/reflectance mode) and Rf values, spectra, λ max and peak area of the resolved bands were recorded. Relative percentage area of each of the bands was calculated from peak areas. The TLC plates were derivatised by spraying with 5% methanolic ferric chloride solution for the detection of phenolic compounds.

Ash value

The proximate parameters of a plant material indicate the nature of the plant constituents. The total ash value of a plant material indicates the amount of minerals and earthy materials in the plant material. The amount of acid insoluble ash indicates the amount of silacious matter in the plant material[12]. The alcohol soluble extractive value indicates the presence of constituents such as flavonoids, alkaloids, steroids and their glycosides; Water soluble extractive value indicates the presence of sugars, acids and inorganic components of a plant material. The proximate parameters as reported [33] for the tuberous roots of H. indicus indicate that the roots possess a higher percentage of water soluble constituents (18.6-18.8% w/w). Being a root material, the acid insoluble ash (15.5-18.8% w/w) was found to be higher.

Extractive value

The extractive values obtained by extraction of the roots with various solvents indicate a higher extractive value with of water[13].

Journal of Chemical Health Risks

www.jchr.org

JCHR (2025) 15(6), 3105-3118 | ISSN:2251-6727

Table 2: Extraction Yield of Different extracts of H. Indicus Roots.

Extract	% Extractive Value	
	Soxhlet Extraction	Cold Extraction
Hexane	4.2	0.2
Petroleum ether	3.25	-
Ether	0.46	-
Benzene	0.4	-
Choloroform	0.42	2.6
Ethanol (90%)	6.25	7.1
Methanol (90%)	9.12	-
Water	6.4	4.2

Elemental analysis

Trace elements present in the methanol extract of roots of H. indicus was analyzed by Atomic Absorption Spectroscopy as reported. The percentage of elements present in the hexane and hydro ethanol extract (90:10 ethanol -water mixture) of roots of H. indicus was analyzed by energy dispersive X-Ray analysis in our laboratory (Instrument make: MIRA3 TESCAN). The nonpolar hexane extract is found to contain almost equal elemental percentage of carbon and oxygen in addition to the elements Mg, Fe, and Ca. This is indicative of the presence of oxygenated low polar molecules in the roots to a greater extent. The respective energy dispersive X-Ray Spectra depict this[14].

Table 3: Percentage of Elements Detected in Root Extracts.

Elements	Xexane Extract (Weights %)	Hydroethanol Extract (Weights %)	Methanol Extract (Weights %)
C	445.36	81.28	-
O	43.56	16.72	-
Mg	1.07	-	-
Si	1.58	0.36	-
Cu	2.56	-	0.85
K	2.36	0.0	-
Ca	0.42	-	-
Fe	2.36	-	10.09
Al	-	0.65	-
Zn	-	-	2.76
Mn	-	-	1.15

Free radical scavenging activity

• *Assay for antiradical activity:* Antiradical activity was measured by a decrease in absorbance at 516 nm of a methanolic solution of coloured DPPH brought about by the sample. A stock solution of DPPH (1.3 mg/ml methanol) was prepared such that 75 µl of it in 3 ml methanol gave an initial absorbance of 0.9. This stock solution was used to measure the antiradical activity[15]. Decrease in the absorbance in the presence of methanolic extract of *H. indicus* root bark at different concentrations was noted after 15 min. EC50 was calculated from % inhibition. Pyrogallol was used as positive control. Suitably diluted stock solution of methanolic extract was spotted on TLC plates and they were developed in different solvent systems for resolving compounds of different polarities as described above. Then they were sprayed with 0.2% DPPH in methanol. Bleaching of DPPH by the resolved bands was observed for 10 minutes and the details recorded[16].

• ***Assay for superoxide radical scavenging activity:***

The assay was based on the capacity of the methanolic extract of *H. indicus* root bark to inhibit formazon for mation by scavenging the superoxide radicals generated in riboflavin-light-NBT system. The reaction mixture contains 50 mM phosphate buffer pH 7.6, 20 µg riboflavin, 12 mM EDTA, NBT 0.1 mg/3ml, added in

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JCHR (2025) 15(6), 3105-3118 | ISSN:2251-6727

that sequence. Reaction was started by illuminating the reaction mixture with different concentrations of sample extract for 90 seconds. Immediately after illumination, the absorbance was measured at 590 nm. Ascorbic acid was used as positive control[p17].

Table 4: TLC figure printing profile of methonolic extract of H. indicus root bark

Scanned at	Solvent system 1			Solvent system 2			Solvent system 2		
	Rf	Lamda max	Relative %	Rf	Lamda max	Relative %	Rf	Lamda max	Relative %
254 nm	0.11	212	13.12	0.15	212	9.01*	0.05	229	3.75*
	0.20	254	2.58	0.25	282	9.02*	0.40	282	28.62/
	0.30	242	15.63	0.42	211	52.28*	0.64	284	29.39*
	0.54	233	3.08	0.55	266	5.72	0.95	280	38.25
	0.62	295	17.73	0.72	300	6.90			
	0.69	293	8.52	0.96	285	11.04			
	0.77	284	3.52						
	0.93	282	35.25						
366 nm**	0.32	317	2.52	57.25	244	9.52			
	0.50	315	57.25	2.74	364	5.72			
	0.64	295	20.95	9.42	265	14.52			
	0.77	288	9.40	2.43	399	22.23			
	0.83	303	2.43	9.92	315	48.56			

*bands which showed positive for Phenolic compounds with methanolic ferric chloride; **all band are fluorescent solvent system 1. Toluene/ethyl acetate (1:1v/v): Solvent system 2. N-butanol/glacial acetic acid/water (4:1:2v/v) Solvent system 3.n-butonal/glacial acetic acid/water/methanol/ethyl acetate (5:1:4:2:4v/v)

• ***Assay for nitric oxide scavenging activity:***

The procedure is based on the principle that, sodium nitroprusside in aqueous solution at physiological pH spontaneously generates nitric oxide which interacts with oxygen to produce nitrite ions that can be estimated using Greiss reagent. Scavengers of nitric oxide compete with oxygen leading to reduced production of nitrite ions[18]. For the experiment, sodium nitroprusside (10 mM) in phosphate buffered saline was mixed with different concentrations of methanolic extract of root bark of *H. indicus* dissolved in methanol and incubated at room temperature for 150 min. The same reaction mixture without the methanolic extract of sample but with equivalent amount of methanol served as control. After the incubation period, 0.5 ml of Griess reagent (1% sulfanilamide, 2% H3PO4 and 0.1% N-(1-naphthyl) ethylenediamine dihydrochloride was added. The absorbance of the chromophore formed was read at 546 nm. Curcumin was used as positive control[19].

Measurement of effect on lipid peroxidation in rat liver homogenate

Rat liver homogenate (10% w/v) was prepared according to the procedure described by Tripathi et al.

Journal of Chemical Health Risks

www.jchr.org

JCHR (2025) 15(6), 3105-3118 | ISSN:2251-6727

(1996). Peroxidation was induced in liver tissue by Iron-ADP complex in the presence of ascorbic acid. The incubation medium constituted 0.5 ml of liver homogenate (10% w/v), 100 µM FeCl3, 1.7 µM ADP, 500 µM of ascorbate and different concentrations of extract in 2 ml of total incubation medium. The medium was incubated for 20 min at 37 °C. Extent of lipid peroxidation was measured by estimation of malondialdehyde (MDA) content (Vani et al., 1997). Results were expressed in terms of decrease in MDA formation by the sample extract. α-tocopherol acetate was used as positive control.

• Assay for phenylhydrazine induced haemolysis of erythrocytes (membrane stabilization study):

20% PCV (packed cell volume) of erythrocyte suspension (from human blood) was prepared according to the procedure described by Hill and Thornalley (1983). The assay was carried out according to the procedure described by Cazana et al. (1990), with certain modifications. In brief the method is as follows: the incubation mixture comprises of 1 ml of phenylhydrazine hydrochloride (0.5 mM), different concentrations of sample extract and 0.1 ml of 20% erythrocyte suspension made to a total volume of 3 ml with phosphate buffered saline (PBS) solution. The mixture was incubated at 37 °C for 1 hour and centrifuged at 1000 g for 10 min[20].

The extent of haemolysis was measured by recording the absorbance of the supernatant at 540 nm. Suitable controls were kept to nullify the effect of solvents and inherent haemolysis. α-tocopherol acetate was used as a positive control for the inhibition of phenylhydrazine induced haemolysis of erythrocytes.

Neuroprotective potential Evaluation:

Acetylcholinesterase (AChE) and Butyrylcholinesterase (BuChE) inhibition assays Based on our exploratory study, we can convey that, methanol extract of H. indicus demonstrated the highest AChE and BuChE inhibitory activity with IC50 of (17.46±0.49 µg/mL) and (31.05±0.39 µg/mL) respectively. Likewise, aqueous extracts too showed equally satisfactory results against AChE (IC50 =21.19±0.4 µg/mL) and BuChE (IC50 =39.23±0.11 µg/mL), while, pet ether extract displayed marginal inhibition potential. Interestingly, a significant correlation was observed between AChE (R=-0.888) and BuChE (R=-0.915) inhibitory activities and concentration of TTRC.

Table 5: Correlation Coefficient between Phytochemical compounds and antioxidant, anti-inflammatory, cholinesterase and MAO-B inhibitory activities of H. indics roots

Assays	TPC		TTC		TFC	
	r	p	r	p	r	p
NO	-0.975	0.032*	-0.969	0.289	-0.979	0.042
DPPH	-0.972	0.676	-0.998	0.0323*	-0.987	0.344
Anti-inflammatory	-0.973	0.042*	-0.998	0.038*	-0.986	0.29*
AChE	-0.972	0.666	-0.888	0.0027**	-0.839	0.989
BuChE	-0.925	0.568	-0.915	0.0442*	-0.890	0.879
MAO-B	-0.882	0.035*	-0.796	0.698	-0.850	0.49*

** indivates P 0.01 and P 0.01. TPC-Total Phenolic Content, TTC-Total Terpenoid content, TFC- Total Flavonoid Content,

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JCHR (2025) 15(6), 3105-3118 | ISSN:2251-6727

Antioxidant activity

The superoxide radical, as a precursor to the more reactive oxygen species is known to be very harmful to cellular components[21]. Hydroxyl radicals are the most reactive species and initiate the peroxidation of cell membranes. The lipid radicals thus generated begin a chain reaction in the presence of oxygen, producing lipid peroxide, which decomposes into aldehydes such as malondialdehyde, which is considered mutagenic and carcinogenic. Plant extract shows inhibition of Fe3+ ADP induced lipid peroxidation and ascorbate system in liver It appears that phenylhydrazine can generate various reactive species[22,23], including superoxide radical, hydrogen peroxide, hydroxyl radical, and phenyl radical, under aerobic conditions. These reactive species can initiate the peroxidation of unsaturated fatty acids in endogenous membrane phospholipids, potentially affecting red cell membrane structure and function. The mentioned methanolic extract was able to inhibit phenylhydrazine- induced hemolysis of erythrocytes, suggesting its ability to scavenge these free radicals and protect against their damaging effects[24]. This is significant because erythrocyte hemolysis in this modelis caused by the generation of different free radicals. One of the most accepted methods of antioxidant activity is DPPH radical scavenging activity assay.

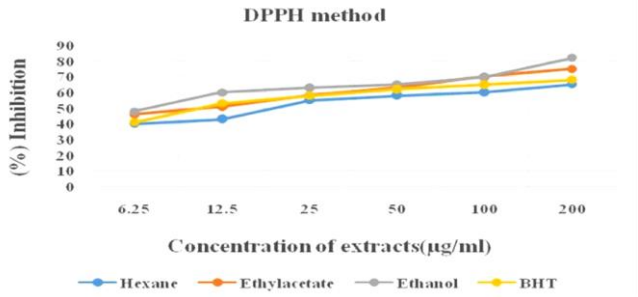

Figure. 1: DPPH method of Antioxidant property of *Hemidesmus indicus* root extracts [11].

Apoptosis activity

H. indicus was found to contain 2-hydroxy-4-methoxybenzaldehyde, 3-hydroxy-4-methoxybenzaldehydeand 2-hydroxy-4-methoxy benzoic acid which induces apoptosis [3,25].

Antiresorptive activity

Hemidesmus indicus appears to be a promising candidate due to its antiosteoclastic activity, which is like that of bisphosphonates but without toxic effects. Its high effectiveness at low concentrations suggests its potential as a therapeutic agent. This makes it worth exploring *Hemidesmus indicus* for its therapeutic potential, either as a standalone treatment or in combination with conventional drugs. Such an approach could potentially improve therapy adherence and have a positive impact on clinical outcomes [25,26].

Indomethacin-Induced Enterocolitis in Rats:

Two days treatment with indomethacin (7.5 mg/kg, s.c.), produced severe inflammation in rat intestine. The middle portion of the small intestine i.e. jejunum and proximal ileum showed more inflammation compared to proximal portion of the small intestine[27]. Caecum was the most severely affected part, showing hemorrhagic spots. The ileum showed many lesions, which were transmural. In between there were skip areas of normal tissue. In some animals the large intestine was found to be affected with hemorrhagic lesions. Evaluation based on macroscopic features showed significantly [28].

Effect of H. Indicus Root Bark extract on macroscopic features in indomethacin induced entero colitis in rats

S. no. Treatment groups Mean of macroscopic scores ± S.E.M 1 Normal or untreated animals. 0 2 Control animals receive only indomethacin (7.5mg/kg) s.c. 8.35 ± 0.57 3 Animals treated with indomethacin (7.5 mg/kg) s.c + lower dose. (TGE) 5.34 ± 0.87* 4 Animals treated with indomethacin (7.5 mg/kg) subcutaneous + higher dose. (TGE) 3.33 ± 0.56x 5 Animals, which will receive Prednisolone (2 mg/kg p.o) and indomethacin (7.5 mg/kg) 1.6 ± 0.22x

Table 6: Effect of H. Indicus Root Bark extract on macroscopic features in indomethacin induced entero colitis in rats

S. No	Treatment Groups	Mean of Macorscopic Scores ± SEM
1	Normal or untreated animals	0

Journal of Chemical Health Risks

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JCHR (2025) 15(6), 3105-3118 | ISSN:2251-6727

2	Control animals receive only indomethacin (7.5mg/kg) s.c.	8.35 ± 0.57
3	Animals treated with indomethacin (7.5 mg/kg) s.c + lower dose. (TGE)	5.34 ± 0.87*
4	Animals treated with indomethacin (7.5 mg/kg) subcutaneous + higher dose. (TGE)	3.33 ± 0.58x
5	Animals, which will receive Prednisolone (2 mg/kg p.o) and indomethacin (7.5 mg/kg)	1.6 ± 0.22x

Table 6 represents significant reduction in macroscopic score value compare to Indomethacin alone. Each value represents mean of macroscopic scores ± S.E.M. (n = 6). Significant in macroscopic score values according to one-way ANOVA followed by Tukey's post-test, P values of *< 0.05 were considered statistically significant. X P < 0.001 compare to indomethacin alone.

The myeloperoxidase assay showed significant increase in MPO activity of positive control group compared to normal untreated group. The drug treated and standard treated group showed significant ($P < 0.01$, $P < 0.001$) reduction in MPO activity compared to the positive control group. MPO activity of the drug treated group was comparable with the standard treated group. The lactate dehydrogenase (LDH) assay showed significant ($P < 0.001$) increase in LDH activity of positive control group compared to normal untreated group. The drug treated and standard treated group showed significant ($P < 0.01$, $P < 0.001$) reduction in LDH activity compared to the positive control group. LDH activity of the drug treated group was comparable with the standard treated group[29].

The Lipid peroxidase (LPO) assay showed significant ($P < 0.001$) increase in LPO activity of positive control group compared to normal untreated group. The drug treated and standard treated group showed significant ($P < 0.01$, $P < 0.001$) reduction in LPO activity compared to the positive control group. LPO activity of the drug treated group was comparable with the standard treated group. Histological examination of positive control group showed advanced lesions as necrosis of even payers patches and fragmentation of nuclei. The drug treated group showed reduced intensity of lesions without any evidence of necrosis, regeneration or inflammatory reaction. Standard treatment showed suppressed inflammatory reaction[30].

Table 7: Effect of H. indicus root bark extract on MPO, LDH and LPO activity in indomethacin-induced enterocolitis

Groups	Treatment Groups	MPO activity (U/g)± S.E.M	LDH activity (U/L) ± S.E.M	LPO Activity (µmol/g) S.E.M
1	Normal or untreated animals	2.16 ±0.34	514.67 ± 6.37	0.16 ±0.03
2	Control animals receive only Indomethacin (7.5mg/kg) s.c.	12.66 ±0.72	1153.67±60.68	0.70 ±0.05
3	Animals treated with Indomethacin (7.5mg/kg) s.c. + lower dose (TGE)	8.34 ±0.84B	908.17 ±75b	0.47 ±0.07c
4	Animals treated with Indomethacin (7.5mg/kg) s.c.+ higher dose(TGE)	4.00 ±1.13C	838.0 ±61a	0.3 ±0.07a
5	Animal treated group, which will receive Prednisolone (2mg/kg p.o) and indomethacin 7.5mg/kg) s.c.	1.68 ±0.21C	555.68 ±100b	100b 0.14 ±0.02b

Journal of Chemical Health Risks

www.jchr.org

JCHR (2025) 15(6), 3105-3118 | ISSN:2251-6727

Each value represents mean of MPO activity (U/g), LDH (U/L), LPO (µmol/g) ± S.E.M. (n = 6). (One-way ANOVA followed by Tukey's post-test)

c. Significant increase in MPO, LDH, LPO activity P

a. Significant decrease in MPO, LDH, LPO activity P

b. Significant decrease in MPO, LDH, LPO activity P

Acetic Acid-Induced Colitis in Rats: Intra-rectal instillation of acetic acid caused inflammatory reaction in the colon[31]. The inflammation covered rectum and distal colon portion. The visible changes included severe epithelial necrosis and ulcerated mucosa. Drug treated and standard treated group showed significantly ($P < 0.001$) lower score values of macroscopic evaluation as compared to positive control group and values obtained for the drug treated group were comparable with the standard treated group

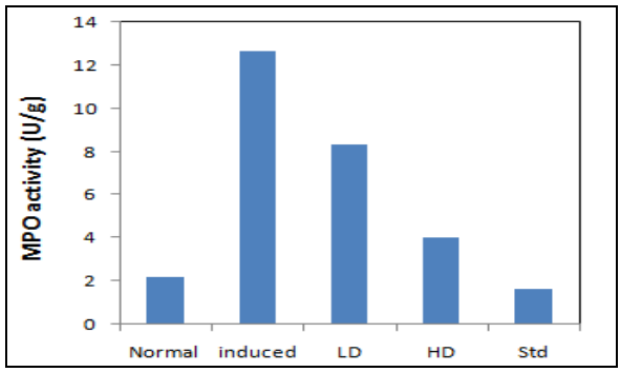

Figure 2: Effect of H. indicus root bark extract on MPO activity in Indomethacin-Induced Enterocolitis in rats

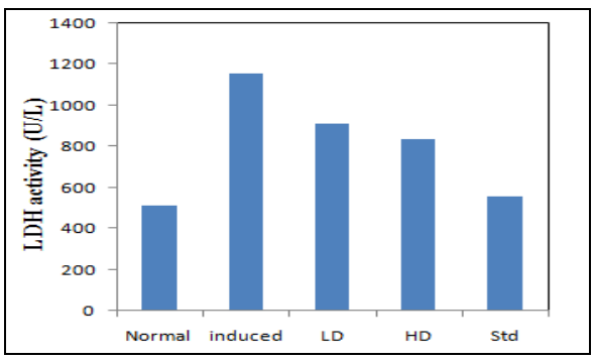

Figure 3: Effect of H. indicus root bark extract on LDH activity in Indomethacin-Induced Enterocolitis in rats

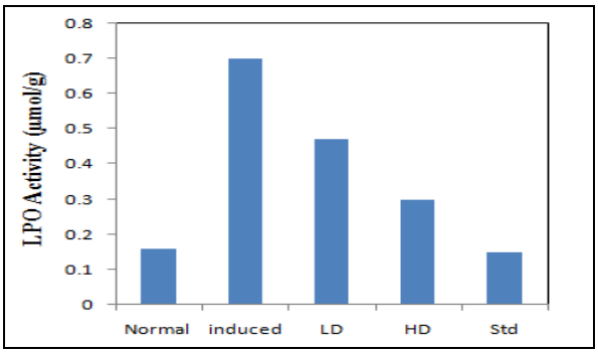

Figure 4: Effect of H. indicus root bark extract on LPO activity in Indomethacin-Induced enterocolitis in rats.

Table 8: Effect of H. indicus root bark methanolic extract macroscopic features in acetic acid induced colitis in rats

S. no.	Treatment groups	Mean of macroscopic
1	Normal	0
2	Positive control – Acetic acid in Saline alone 2ml (4%,v/v) (once, intra-rectally)	10.16± 0.41
3	Methanolic extract of *H. indicus root bark*+ Aceticacidinsaline2ml (4%, v/v) (once, intra-rectally)[TGE]	6.68± 0.82*
4	Methanolic extract of *H. indicus root bark*+ Aceticacidinsaline2ml (4%, v/v) (once, intra-rectally)[TGE]	2.84 ± 0.47x
5	Standard (500mg/kg,p.o.) + Acetic acid in saline 2 ml) (once, intra-rectally)	2.16± 0.18x

Journal of Chemical Health Risks

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JCHR (2025) 15(6), 3105-3118 | ISSN:2251-6727

Table 8 represents significant reduction in macroscopic score value compare to acetic acid alone. Each value represents mean of macroscopic scores ± S.E.M. (n = 6). Significant in macroscopic score values according to one-way ANOVA followed by Tukey's post-test, P values of < 0.05 were considered statistically significant. cP < 0.001 compare to acetic acid alone

The myeloperoxidase assay showed significant (P < 0.001) increase in MPO activity of positive control group compared to normal group. The drug treated and standard treated groups showed significant (P < 0.01, P < 0.001) decrease in MPO activity compared to positive control group. The lactate dehydrogenase (LDH) assay showed significant (P < 0.001) increase in LDH activity of positive control group compared to normal group. The drug treated and standard treated groups showed significant (P < 0.01, P < 0.001) decrease in LDH activity compared to positive control group[32].

The lipid peroxidase (LPO) assay showed significant (P < 0.001) increase in LPO activity of control group compared to normal group. The drug treated and standard treated groups showed significant (P < 0.01, P < 0.001) decrease in LPO activity compared to positive control group.

Histological examination of control group showed massive necrosis of the mucosa and submucosa. Payers patches appeared distorted with karyohexis and karyolysis. Drug treated group showed mild lesions, regeneration and inflammatory reaction. The standard treated group showed suppressed inflammatory reaction[33].

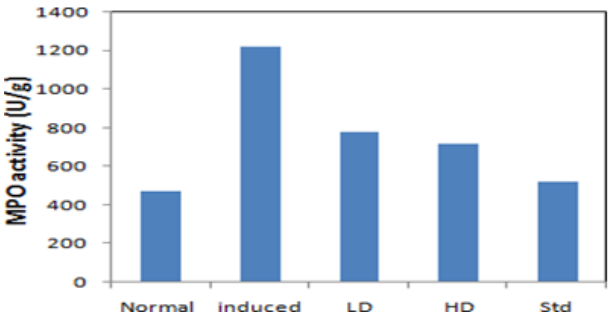

Figure 5: Effect of H. indicus root bark extract on LDH activity in acetic acid-induced entero colitis in rats

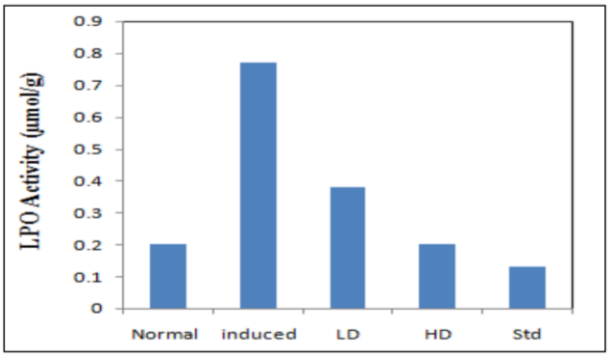

Figure 6: Effect of H. Indicus root bark extract on LPO activity in acetic-induced enterocolitis in rats

Table 10: Effect of Methanolic extract of H. Indicus root barkon MPO, LDH AND LPO activity in acetic acid-induced colitis in rats

Groups	Treatment groups	MPO activity (U/g) ±S.E.M	LDH activity (U/L) ± S.E.M	LPO Activity (µmol/g)S.E.M
I	Normal	2.67±0.21	472.33±46.22	0.20±0.04
II	Positive control- Acetic acid in salinealone 2 ml (4%,v/v) (once, intra-rectally)	12.83±0.87	1221.83±55.88	0.77±0.05
III	Methanolic extract of Tectonagrandis + Acetic acid in saline 2ml (4%,v/v) (once,intra-rectally [TGE]	7.83±0.87	778.50±96.20	0.38±0.09
IV	Methanolic extract of Tectonagrandis + Aceticacidin saline 2ml (4%,v/v) (once, intra-rectally) [TGE]	2.50±0.56	721.50±95.91	0.20±0.03

Journal of Chemical Health Risks

www.jchr.org

JCHR (2025) 15(6), 3105-3118 | ISSN:2251-6727

V	Standard (500 mg/kg,p.o.) + Acetic acid in saline 2ml (4%,v/v) (once ,intra- rectally)	1.50±0.22	523.33±83.62	0.13±0.02

Each value represents mean of MPO activity (U/g), LDH (U/L), LPO (µmol/g) ± S.E.M. (n = 6). (One-way ANOVA followed by Tukey's post-test) c. significant increase in MPO, LDH, LPO activity P

TGE was evaluated for its in vitro COX-1 and COX-2 inhibitory activities by using colorimetric COX (human ovine) inhibitor screening assay kit. The results showed that TGE inhibits COX-1 about 43.17% and that of COX-2 is 48.67% see 11 . Prostaglandins inhibition was studies by inhibition of Castor oil induced diarrhea in rats. Administration of Castrol induced diarrhea around 9 fecal drops with total weight of 5.5 grams in 4 h[34]. This was reduced in both test and standard groups. Indicating TGE produces action by inhibiting the synthesis of prostaglandins see Table 12.

Table 11: Effect of KEE on Cox Inhibitory Activity

Extract	Inhibition of COX-1 (%)	Inhibition of COX-2 (%)
TGE	43.17	48.67

Table 12: Determination of Prostaglandins Inhibition

Groups	Treatment and dose	Mean frequency of diarrhea ± SEM	Mean no. of fecal drops ± SEM	Mean wt. of feaces ± after 4 hrs (gm)
1	Control (0.5 ml of DI water)	7.17±1.47	9.67±1.51	5.17±1.17
2	Aspirin150 mg/kg	1.67 ±0.82	3.67±1.21	3.33±1.86
3	TGE	1.33±0.52	4.67±3.08	3.17±1.33

Bark extract of H. indicus root bark Linn. is screened for inflammatory bowel disease. Various parts of the plant have showed activities like astringent, depurative, diaphoretic, diuretic expectorant, febrifuge, odontalgic and ophthalmic[35]. The aerial part of this plant has been reported to contain pyrrolizidine alkaloids and flavonoids . The aqueous extract of the aerial parts have shown antimicrobial activity. The plant is astringent, sweet, thermogeic, ntipyretic and antiasthmatic. Aqueous and methanolic extract of H indicus leaves have been shown to progressively reduced rat paw edema induced by sub plantar injection of albumin, which suggest that this extract might have antiinflammatory principals. The bark extract of H. indicus root barkLinn. has antioxidant and antiproliferative effects on human cancer cells . In a study H. indicus root barkLinn. Stem bark extracts showed significant in vivo analgesic and antiinflammatory activities. The models used for study of IBD are acetic acid induced ulcerative colitis and indomethacin induced enterocolitis. Intrarectal instillation of acetic acid in rats affected only the distal colon portion. The inflammation was not transmural. Massive necrosis of mucosal and submucosal layers was observed. This model shares many of the histologic features of ulcerative colitis in human beings including mucosal edema, neutrophil infiltration of the mucosa and submucosal ulceration. The mechanism by which acetic acid produces inflammation appears to involve the entry of the protonated form of the acid into epithelium, where it dissociates to liberate protons causing intracellular acidification that most likely accounts for the epithelial injury observed. The inflammatory response initiated by acetic acid includes activation of cyclooxygenase and lipooxygenase pathways and generation of inflammatory mediators like prostaglandins and leukotrienes. Excess production of reactive oxygen metabolites e.g. superoxide, hydroxyl radical, hydrogen peroxide, hypochlorous acid and oxidant derivatives such as N-chloramines are detected in inflamed mucosa and may be pathogenic in IBD. Also, there is an increase in proinflammatory

Journal of Chemical Health Risks

www.jchr.org

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cytokine TNF -α production in colonic mucosa after acetic acid instillation. Indomethacin, a nonselective COX inhibitor produces enterocolitis in rats on sub cuteneous administration which is characterized by linear ulceration, thickening and transmural inflammation. The mechanism of indomethacin induced enterocolitis have not been fully illustrated, but previous reports suggests that, inhibition of protective prostaglandins PGE1, PGE2 and prostacyclin (PG12) may be one of the mechanism by which indomethacin induces injury. In addition, bacteria and bacterial products, biliary secretion and food intake have been demonstrated to be important for the development of the intestinal lesions. The treatment with bark extract H. indicus root barkLinn. has shown a decrease in the macroscopic scores for the inflammation. Since the intestine is in a constant state of controlled inflammation, thus amplification of the inflammatory response activates infiltration of inflammatory cells that triggers pathological responses and symptoms of IBD . Our study showed that acetic acid raised the levels of colonic MPO, indicating infiltration of neutrophils and perturbation of the inflammatory system. This fact is documented in animal models, with IBD. H. indicus root bark Linn. bark extract ameliorated neutrophil infiltration as evidenced by suppression of colon MPO and improvement of histological features. Histopathology examination of drug treated group revealed less damage compared to control group. A significant decrease in MPO activity was also observed. All these observations support the findings that the barks extract of H. indicus root barkLinn. was able to offer significant protection in both the models studied. Acetic acid-induced colitis and indomethacin-induced enterocolitis simulate two different disease conditions, which are ulcerative colitis and Crohn's disease respectively. On this basis we can say that the constituents extract under study may be useful in treating UC as well as CD in humans. The prednisolone treatment has shown significant protection in both the animal models under our study. The bark extract H. indicus root barkLinn. was found comparable with standard drug. The role of oxygen-derived free radicals, such as hydroxyl radical and superoxide radical, in the inflammatory process is well known. It is also generally assumed that most of the antioxidants possess anti-inflammatory effect. Bark extract of H. indicus root barkLinn. may account for the observed anti-inflammatory properties.

CONCLUSION: These Phytochemical compounds of Hemidesmus indicus root bark have been found to induce a wide spectrum of activities such as reduction in oxidative stress, suppression of inflammation, and cell proliferation and modulating numerous signal transduction pathways. On the basis of the results obtained in the present study, it is concluded that an 70% methanolic extracts of roots of Hemidesmus indicus exhibits high antioxidant and free radical scavenging activities. Bark extract of H. indicus root barkLinn. has potent protection against inflammatory bowel activity in both indomethacin induced enterocolitis and acetic acid induced ulcerative colitis. Collectively, these findings indicate that the anti-oxidant effect of fraction may be an important contributor to its anti-inflammatory activity in IBD. This present investigation has also opened avenues for treatment of IBD from the title plant.

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