



## Research Article

### EVALUATION OF ETHANOLIC EXTRACT OF *HEMIDESMUS INDICUS* IN COMBINATION WITH *OCIMUM BASILICUM* FOR DEXTRAN SULFATE SODIUM INDUCED COLITIS IN MICE

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#### ABSTRACT

Inflammatory bowel disease (IBD) is a chronic intestinal disease which mainly involves Ulcerative colitis and Crohn's disease. Our study shows mostly the Dextran Sodium Sulphate induced colitis model. Ulcerative colitis is the long-lasting inflammation that is primarily caused due to immune dysregulation, microbiota and other genetical and environmental factors. Dextran sodium sulphate induces the colitis by damaging the intestinal epithelial barrier. In the present study, *Hemidesmus indicus* and *Ocimum basilicum* ethanolic extracts showed the anti-inflammatory activity at a dose 250-500 mg/kg and compared with standard drug sulfasalazine. Among VI groups, group-I animals served with saline, group –II animals are given only Dextran Sodium Sulphate, other groups along with Dextran sodium sulphate they receive ethanolic extracts at different doses according to body weights, the study was conducted at 16 days at the end of the day the animals were sacrificed by giving ether, colon lengths and weights were measured, for group –II animals there is decrease in colon length and weight and also shows the rectal bleeding and slight lesions. Whereas other groups don't show any sores and rectal bleeding. Based on this we confirm the anti-inflammatory activity. After identification of Group – II animals lesions we understood chemoprotective effect against Dextran Sodium Sulphate induced ulcerative colitis.

**Keywords:** Dextran sulfate sodium, Inflammatory bowel disease, Ulcerative colitis, *Hemidesmus indicus*, *Ocimum basilicum*

#### INTRODUCTION

Inflammatory bowel disease (IBD) is a multifactorial disease. It involves acute and chronic inflammation of all parts of your digestive tract. The two conditions that include inflammation of the intestine are ulcerative colitis and Crohn's disease<sup>1,2</sup>. Both are idiopathic chronic diseases of the gastrointestinal tract. Mainly the chronic inflammation is due to the imbalance of the intestinal microbiota. Ulcerative colitis is an inflammatory bowel disease that causes long-lasting inflammation and sores (ulcers) in the innermost lining of your large intestine (colon) and rectum<sup>3-5</sup>. Crohn's disease is an IBD that causes inflammation of the lining of your digestive tract. In Crohn's disease, inflammation often spreads deep into affected tissues. The inflammation can involve different areas of the gastrointestinal tract the large intestine, small intestine or both<sup>6-9</sup>.

*Hemidesmus indicus*, Indian sarsaparilla is a species of plant that is found in South Asia. It is a slender, laticiferous, twining, sometimes prostrate or semi-erect shrub. Roots are woody and aromatic. The roots of *Hemidesmus indicus* contain hexatriacontane, lupeol, octacosanoate,  $\alpha$ -amyrin,  $\beta$  amyrin, acetate and sitosterol<sup>10</sup>. It also includes new coumarino-lignoid-hemidesminine, hemidesmin I and hemidesmin II50, six pentacyclic triterpenes including two oleanenes, and three ursenes. It contains essential oil, Starch, Coumarin, Tannic acid, Triterpenoid saponins. The *Hemidesmus indicus* have Bacteriostatic, anticancer, antiviral, antilithic, hypotensive, antifungal, antibacterial, anti-inflammatory, spasmodic activities, etc<sup>11</sup>.

Basil (*Ocimum basilicum*) also called the "king of herbs" and the "royal herb." Basil is possibly native to India. The strong clove scent of sweet basil is derived from eugenol, the same chemical as actual cloves. The citrus scent of lemon basil and lime basil reflects their higher portion of citral, which causes this effect in several plants including lemon mint, and of limonene, which gives actual lemon peel its scent. Licorice basil contains anethole, the same chemical that makes anise smell like licorice. Basil seeds have antioxidant, anticancer, antiviral, antibacterial, antimicrobial, antispasmodic, antiseptic, antifungal, carminative, galactagogue, and stomachic properties, anti-inflammatory properties. The carminative features are useful in providing relief in a wide range of digestive issues like flatulence, stomach cramps, indigestion, constipation, etc<sup>12-14</sup>. In this present research, the author mainly identified drug for inflammatory bowel disease by using Dextran sodium sulphate induced colitis model and also to study the effect of extracts of *Hemidesmus indicus* roots and *Ocimum basilicum* seeds powder against Dextran sodium sulphate induced colitis in mice.

#### MATERIALS AND METHODS

##### Preparation of Plant Extract of *Hemidesmus indicus* root powder

The plant materials were obtained were authorized by taxonomist Dr. M.Vijaya lakshmi and given as a free gift by Dr. M.Vijaya lakshmi, Department of Botany, University College of Pharmaceutical Sciences, Guntur.

**Soxhlet Extraction Method:** Fresh roots of *Hemidesmus indicus* were collected washed with water and then shade dried and powdered in a mechanical grinder. The powdered material was subjected to successive extraction in a Soxhlet extraction process<sup>15</sup>. The powdered material was packed and preceded for the removal by using ethanol<sup>16</sup>. The extracts were concentrated and air dried. Final yield was 10 gm of ethanolic extract of the plant.

**Preparation of Plant Extract of *Ocimum basilicum* seeds powder**

**Soxhlet Extraction Method:** Fresh seeds of *Ocimum basilicum* were collected washed with water and then dried and powdered in a mechanical grinder. The powdered material was subjected to successive extraction in a Soxhlet extraction process. The powdered material was packed and preceded for the removal by using ethanol. The extracts were concentrated and air dried. Final yield was 5 gm of ethanolic extract of the plant<sup>17</sup>.

The ethanolic extract obtained from the above extraction processes was analyzed for different phytoconstituents present in this by the method of qualitative phytochemical analysis. After the tests are conducted, it was found that these extracts contain alkaloids, carbohydrates, cardiac glycosides, anthraquinone glycosides, gums and mucilage, proteins and amino acids, tannins and phenolic compounds, steroids and sterols, triterpenoids, saponins and flavonoids<sup>18</sup>.

**Selection of the animal species:** The preferred rodent species is mice, although other rodent species may be used. Animals of either sex can be chosen we selected female mice. Each animal, at the commencement of the dosing, were between 7 and 9 weeks old. The Temperature in the experimental room was maintained at 22°C (±3°C). Although the relative humidity was kept at least 30% and preferably not exceeded 70%. Lighting was artificial, the sequence being 12 hours light, 12 hours dark<sup>19,20</sup>. Animals were purchased from Mahaveer Enterprises, Hyderabad. The study was approved by IAEC, CPCSEA, New Delhi (007/IAEC/NCPA/M.Pharm/2016-2017).

Animals were grouped in the following order

Group 1	Group 2	Group 3	Group 4	Group 5	Group 6
10	10	10	10	10	10
Animals	Animals	Animals	Animals	Animals	Animals

**Animals selection:** The animals were randomly selected, marked to permit individual identification, and kept in their cages for at least 5 days prior to dosing to allow for acclimatization to the laboratory conditions.

**Procedure:** The primary objective is to identify drug for IBD by using Dextran Sodium Sulphate induced colitis. The animals used for the experiment were healthy and active. On the starting day of the experiment the body weights of the animals were checked and fed with feed and drinking water / saline. Animals were divided into 6 groups, according to body weights each group containing 10 animals;

- 1) Group I: -ve control animals treated with saline.
- 2) Group II: +ve control animals treated with Dextran Sodium Sulphate.
- 3) Group III: animals treated with standard drug Sulfasalazine, along with Dextran Sodium Sulphate.
- 4) Group IV: animals treated with ethanolic extract of *Hemidesmus indicus* (250 mg/kg) along with Dextran Sodium Sulphate.

- 5) The Group V: animals treated with ethanolic extract of *Hemidesmus indicus* (500mg/kg) along with Dextran Sodium Sulphate.
- 6) The Group VI: animals treated with ethanolic extract of *Hemidesmus indicus* 500mg/kg, *Ocimum basilicum* of 500mg/kg, along with Dextran Sodium Sulphate.

All the animals were treated with standard and test drugs for 16 days and during the study body weights, and abnormal signs and symptoms were observed.

**Statistical Analysis:** The statistical significance of the treatments was determined by one-way ANOVA using Graph Pad Prism 7. A \*p value of < 0.05 at 95% confidence limit was considered for statistical significance.

**RESULTS**

Daily body weights were checked before administration of drugs and extracts for 16 days. There is an increase in body weights in all groups except in Group -2 animals. The following graph shows the changes in the body weights between groups

At the end of the study, after completion of 16 days, the animals were sacrificed by giving anesthesia. We have observed and noted the colon length, colon weight, and gross score are presented in tables and figures.

**Scoring System**

- Stage 0: No epithelial damage.
- Stage1: Mild epithelial damage with traces of blood in stools.
- Stage2: Moderate epithelial damage with blood in stools.
- Stage3: Severe epithelial damage with bloody stools.
- Stage4: Loss of epithelial tissue with stools leads to death.

**DISCUSSION**

Now a day's inflammatory bowel disease is the major problem in humans due to various reasons. Our primary objective is to identify drug for inflammatory bowel disease by using Dextran sodium sulphate induced colitis model. New findings show Human TGF (tumor growth factor)-β1 deficiency causes severe inflammatory bowel disease and encephalopathy<sup>21</sup>, so by inhibition of TGF we can suppress IBC. In the present study, the extracts were given in order to reduce the levels of TGF. During the study Group – II animals showed rectal bleeding after 10 days but whereas another group of animals don't show any signs, only Group – II animals fecal matter showed bloody lesions which did not observe for any other groups. This indicates the clear inflammatory bowel disease because of so many pro-inflammatory factors<sup>22</sup>. At the end of the study on the 16<sup>th</sup> day all the animals were sacrificed, removed the fecal matter from the colons and measured the colon lengths and the gross observation of colon tissue was closely observed for colon lengths and weights. . Colon lengths also showed a significant difference compared with Group – II animals. The treated group animals showed normal colon length and weight which indicated that the extracts protected the colon from inflammatory bowel disease. Similarly, Glycyrrhizae Radix (GR) is a Korean traditional herb medicine that is widely used in clinical health care also showed protection against inflammatory bowel disease<sup>23</sup>. This anti-inflammatory effect is probably due to the inhibition of pro-inflammatory factors such as TNF and others. So both *Hemidesmus indicus* and *Ocimum basilicum* may be useful for treating inflammatory bowel disease. The limitations were its difficult to identify specific proinflammatory factor responsible for the mechanism of action. So, it is difficult to study all the levels of proinflammatory factors.

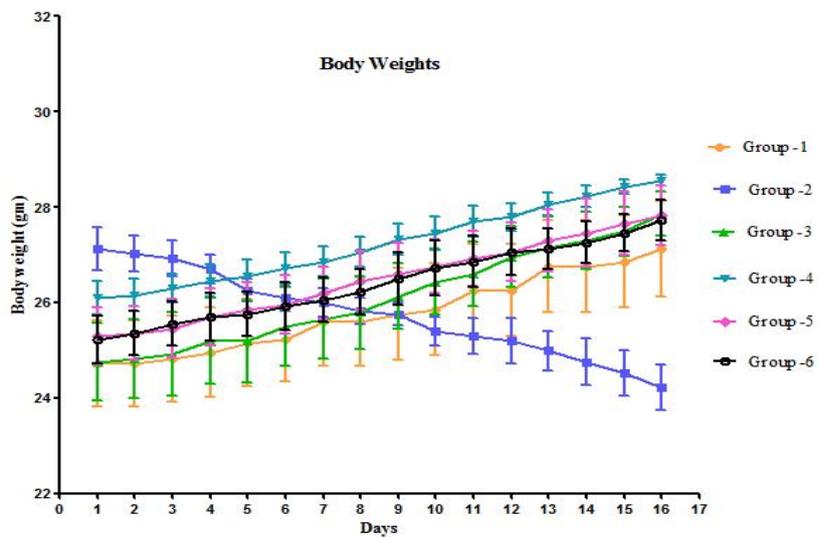


Figure 1: Body weights of swiss albino mice

Table 1: Colon lengths, colon weights and gross score after completing the study

Groups	Animal No	Gross Score	Colon Length (cm)	Colon Weight (gm)
Group 1	1	NL	11.5	3.2
	2	NL	11.5	3.2
	3	NL	11.5	3.2
Group 2	4	3	9.3	1.5
	5	3	9.3	1.5
	6	3	9.3	1.5
Group 3	7	NL	11.5	3.2
	8	NL	11.5	3.2
	9	NL	11.5	3.2
Group 4	10	NL	11.5	3.2
	11	NL	11.5	3.2
	12	NL	11.5	3.2
Group 5	13	NL	11.5	3.2
	14	NL	11.5	3.2
	15	NL	11.5	3.2
Group 6	16	NL	11.5	3.2
	17	NL	11.5	3.2
	18	NL	11.5	3.2

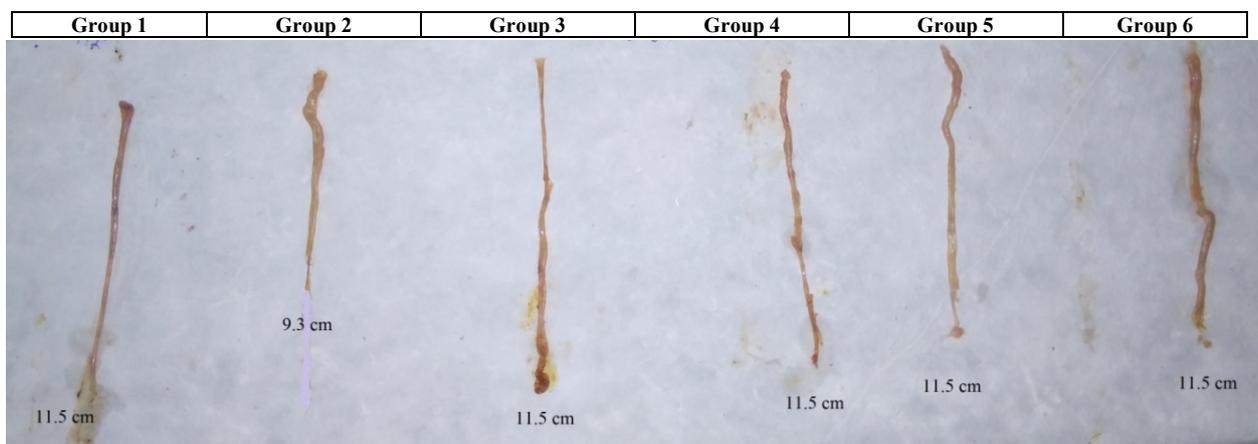


Figure 2: Colon lengths measured at the end of the study

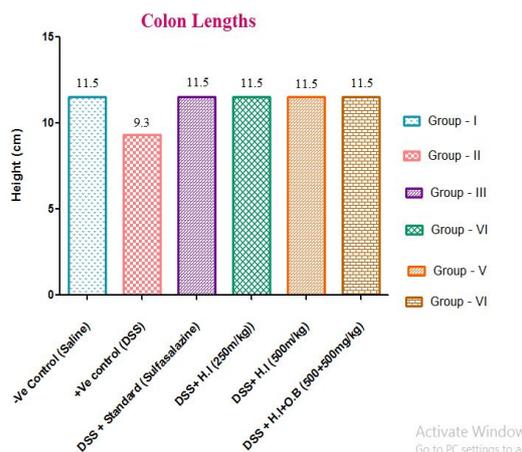


Figure 3: Colon lengths measured at the end of the study

The statistical significance between the treatments was determined by one-way ANOVA using Graph Pad prism software. A \*p value of < 0.05 at 95% confidence limit was considered statistically significant (when compared with the control group).

## CONCLUSION

Both *Hemidesmus indicus* (H.I) and *Ocimum basilicum* (O.B) extracts show the similar response when compared to the standard drug sulfasalazine. These two extracts at a dose of 500mg/kg show the anti-inflammatory activity, and there is no lesions and no rectal bleeding in animals. By this observation, we have concluded the combination of both extracts shows the excellent anti-inflammatory activity. This gives really good insight into herbal medicines activities in combination for the inflammatory bowel disease.

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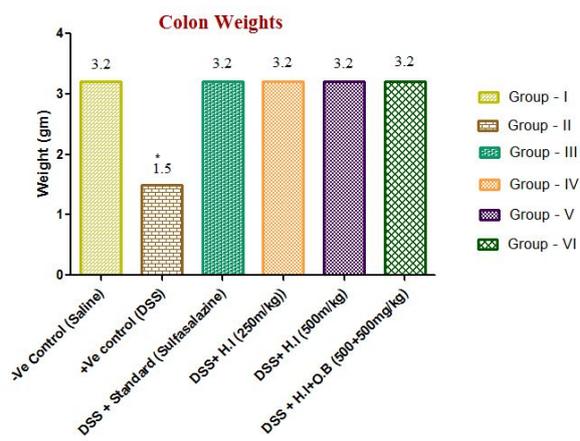


Figure 4: Colon weights measured at the end of the study

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