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Original Research Article

Protecting Consequence of Polyherbal Plant Extracts on Indomethacin induced Enterocolitis in Rats

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Abstract: Inflammatory Bowel Disease refers to a collective term that encompasses various chronic, non-specific disorders of the gastrointestinal tract, including Ulcerative Colitis (UC) and Crohn's Disease (CD). UC is limited to the rectum and colon, impacting both the mucosa and submucosa. The current study aimed to explore the in-vivo intestinal anti-inflammatory properties of a polyherbal plant extract, which is a blend of the roots of Hemidesmus indicus and the seeds of Centratherum anthelminticum. Inflammation was effectively induced through intra-rectal administration of 4% glacial acetic acid in albino Wistar rats via the intra-rectal route to evaluate the acute intestinal anti-inflammatory effects. The positive control group received only the vehicle, while other groups were administered oral treatments with two different doses (500 mg/kg and 1000 mg/kg body weight) along with Prednisolone (2 mg/kg body weight). The animals treated with the polyherbal plant extracts exhibited a significant reduction in colon scores compared to the positive control group. There was a notable decrease in Myeloperoxidase and Lipid Peroxidation levels when compared to the positive control group. The treatment with polyherbal plant extracts is likely responsible for the significant intestinal anti-inflammatory effects observed, potentially through an antioxidant mechanism.

Keywords: Ulcerative colitis, Hemidesmus indicus, Centratherum anthelminticum, Myelo peroxidation, Lipid peroxidation, Poly herbal Plant Extracts.

INTRODUCTION

Inflammatory bowel disease (IBD), clinically defined as ulcerative colitis and Crohn's disease, is a chronic inflammatory condition of the colon and rectum. Despite the fact that the disease's etiology is still unknown, potential etiologic factors include environmental factors, immunological abnormalities, and genetic influences. The role of reactive oxygen species (ROS) in the pathogenesis of IBD has been emphasized in recent years [1–3]. The literature's current evidence strongly indicates a cascade of free radical products, followed by lipid peroxidation, which lowers the cell's antioxidant capability and causes inflammation in the colon. In addition, granulocytes in the inflamed mucosa in IBD have been shown to release a variety of inflammatory mediators, including lysosomal enzymes, reactive oxygen species (ROS), and arachidonic acid metabolism byproducts. 1 [4-5]. The gastrointestinal tract and the colonic mucosa, respectively, may be affected by inflammatory bowel disease (IBD), which is a chronic, remitting, or progressive inflammatory illness linked to a higher risk of colon cancer. The genetic basis of IBD has long been understood, and it is probable that it involves an immune system reaction to certain environmental factor(s). The significance of environmental variables in disease pathophysiology is further emphasized by the discordance of IBD among monozygotic twins and the emergence of IBD in immigrants to high prevalence nations and in countries that are undergoing fast Westernization [6].

The twining shrub known as Hemidesmus indicus. R. Br. has roots that have been used in traditional, ayurvedic, and unani medicine and are recommended for treating illnesses/disorders that affect the respiratory system, the skin, the gastrointestinal tract, the haemopoetic system, infections, and inflammatory diseases. A literature review found that it had been thoroughly researched in order to support its usage in folklore and the traditional medical system. Some of the most significant pharmacological features documented are anti-inflammatory, cardioprotective,

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nephroprotective, and hepatoprotective [8-9]. The seeds of Centratherum anthelminticum (L) Kuntze are widely used in indigenous medicine to treat a variety of conditions. Its numerous applications include its beneficial uses in treating intestinal colic, ulcers, and inflammatory edema. In light of the lack of research documenting the efficacy of alcoholic extract of Centratherum anthelminticum seeds in IBD, despite its reported flavonoid content [12] and acute and sub-acute anti-inflammatory effect, [11] the current study intends to evaluate its potential protective effect.

MATERIALS AND METHODS

Plant Material and Extraction

The methanolic extract of the polyherbal plant extract (Centratherum ant anhelminticum seeds and Hemidesmus indicus roots) stored at 40C in an airtight container in the refrigerator's door refrigerator area until future use. Plant extract was administered at a ratio of 1:1.

Experimental animals

Selection of Test animals: Healthy, Albino Wistar rats (either sex) weighing between 170-200 gms randomly assigned for.

Pharmacological investigations

Enterocolitis in Rats caused by Indomethacin: The male Wistar albino rats (170–230 g) were divided at random into five groups of six animals each. The five groups were as follows: Group 1 was made up of normal or untreated animals; group 2 was the positive control group, which only got indomethacin (7.5 mg/kg) subcutaneously; group 3 was the treated group, which received indomethacin (7.5 mg/kg) subcutaneously along with a lower extract dose; group 4 was the treated group, which received indomethacin (7.5 mg/kg) subcutaneously along with a higher extract dose; and group 5 was the standard group, which received indomethacin (7.5 mg/kg) subcutaneously along with prednisolone (2 mg/kg p.o). On the 8th and 9th day of treatment, animals pretreated for 7 days with PHPE extract will receive Indomethacin (7.5 mg/kg, s.c.). The extract will be given up to the 11th day. The animals will be slaughtered by cervical dislocation on the 12th day.

Dissection, scoring and estimations

The abdomen was opened at the conclusion of the experiment, revealing the ileum, colon, and caecum. A longitudinal incision was used to open the colon's distal 8 cm. Following a colon cleanse to remove fat and mesentery, the colon was divided into three pieces for macroscopic, microscopic, and biochemical analysis of the damage.

Experimental animals with an acute model of colitis were used to score inflamed colons [13]. An observer, unaware of the treatment regimen, performs a macroscopic evaluation of the colon damage, looking for visible injuries as outlined by Bell *et al.,*

Biochemical estimations

The colonic tissue sample will be homogenized, centrifuged, and the resulting supernatant will be used to measure the colonic MPO activity [14] in order to quantify inflammation) MDA [15]. as previously mentioned.

Determining the amount of myeloperoxidase [MPO] in the tissue of the colon.

As a sign of neutrophil infiltration, MPO activity was measured as follows: Pieces of inflamed tissue (2 cm from the rat colon and 2 cm from the rat ileum) were rinsed with ice-cold saline, blotted dry, weighed, and excised. A tissue homogenizer was used to homogenize the minced tissue in 10 volumes of ice-cold potassium phosphate buffer (pH 7.4). At 4°C, the homogenate was centrifuged for 30 minutes at 10,000 rpm. The reaction was started by combining phosphate buffer with the supernatant that was gathered with 300 mM H2O2 and 660 mg/ml of o-phenylenediamine. For five minutes, absorbance was measured at 492 nm every 30 seconds. The alteration in absorbance per minute by 1.0 at room temperature in the last reaction is considered to be one unit of MPO activity.

Calculation of MPO activity

$$\text{MPO activity (U/g)} = \frac{X}{\text{Wt. of the piece of tissue taken}}$$

$$\text{Where } X = \frac{10 \times \text{change in absorbance per minute}}{\text{Volume of supernatant taken in the final reaction}}$$

Estimation of lipid peroxidation in the Colon tissue.

The thiobarbituric acid reaction method, as detailed by Ohkawa *et al.,* was used to determine the amount of lipid peroxides. To 0.2 ml of the sample, 0.2 ml of SDS, 1.5 ml of acetic acid, and 1.5 ml of TBA were added. The mixture was heated for an hour at 95°C in a water bath after being diluted with water to a volume of 4 ml. 1 mL of water and 5 mL of an n-butanol/pyridine combination were added after cooling, and the mixture was vigorously shaken. The organic layer was collected

after 10 minutes of centrifugation at 4000 rpm, and its absorbance was measured at 532 nm. The amount of lipid peroxides was measured as n moles of MDA produced per gram of wet tissue.

Reagents	Sample	Blank
SDS	0.2 ml	0.2 ml
Supernatant	0.2 ml	……
DDW	1.6ml	1.8 ml
Acetic acid	1.5 ml	1.5ml
TBA	1.5 ml	1.5ml
n-butanol/pyridinemix	5ml	5ml

$$\text{Concentration of MDA} = \frac{\text{Absorbance at 532 nm}}{\text{Lx?}} \times D$$

Where,
L: lightpath (1cm).
?: Extinction coefficient1.56x105M-1. Cm-1.

$$\text{D: dilution factor} = \frac{\text{Total volume (10ml)}}{\text{Volume of the sample (0.2ml)}}$$

STATISTICAL ANALYSIS

Using Graph Pad Prism 8.0, all values were represented as mean±SEM and statistically analyzed using one-way analysis of variance (ANOVA) and Tukey's post test.

A statistically significant value for P was anything less than 0.5.

RESULT & DISCUSSION

Protective Effect of Extract on Indomethacin Induced Enterocolitis

Effect of PHPE treatment on histopathological changes / macroscopic changes of inflamed GIT

Table 1: Effect of PHPE treatment on histopathological changes / macroscopic changes of inflamed GIT

Group	Treatment	Scoring of various parts of GIT		
		Colon	Caecum	Ileum
I	Normal	0.0±0.0	0.0±0.0	0.0±0.0
II	Positive Control	8.35 ± 0.57	7.40±0.40	4.00±0.31
III	PHPE Lower Dose	5.34 ± 0.87*	4.20±1.53*	3.2±1.5,NS
IV	PHPE Higher Dose	3.33 ± 0.58^{x}	2.80±0.48^{x}	2.40±0.40$^{\beta}$
V	Prednisolone (2mg/kg)	1.60 ± 0.22 ^{x}	1.40±0.24 ^{x}	1.20±0.20*

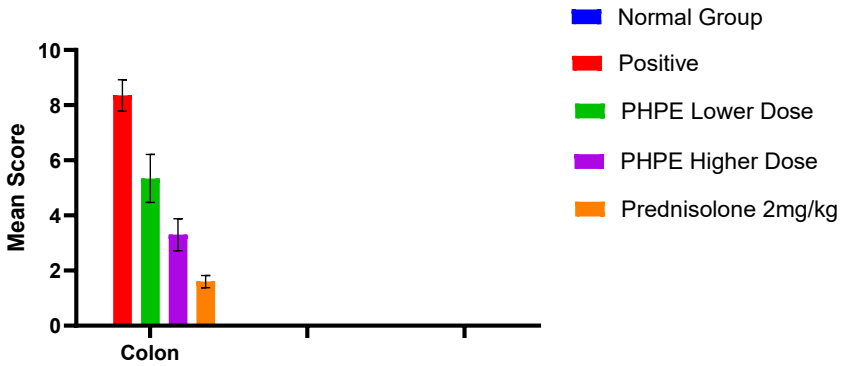


Fig. 1: Effect of extract treatment on macroscopic scoring of colon

All value are mean ± SEM of n=5 One way ANOVA followed by Tukey's post-test.

Significant difference between score of colon of normal and positive control group was recorded (P<0.001). *P<0.05 Positive Control Vs PHPE (Lower Dose), x P<0.001Positive Control Vs PHPE (Higher Dose) & Prednisolone (2 mg/kg)

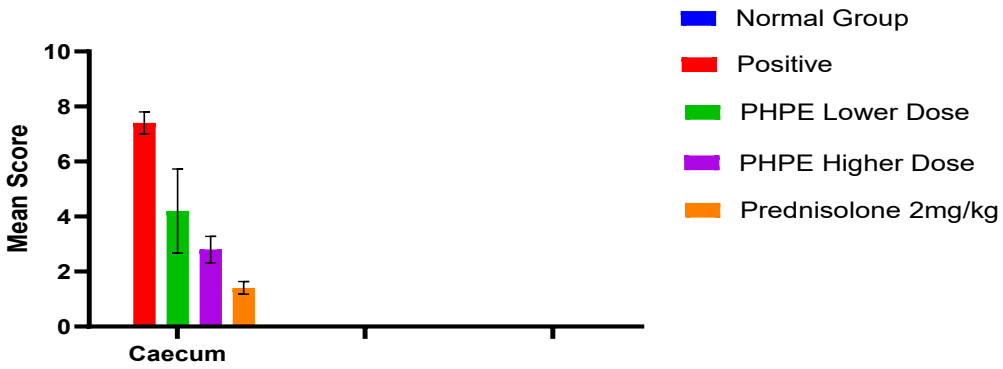


Fig. 2: Effect of extract treatment on macroscopic scoring of Caecum

All value are mean ± SEM of n=5 One way ANOVA followed by Tukey's post-test.

Significant difference between score of caecum of normal and positive control group was recorded (P<0.001). *P<0.05 Positive Control Vs PHPE (Lower Dose), x P<0.001Positive Control Vs PHPE (Higher Dose) & Prednisolone (2 mg/kg)

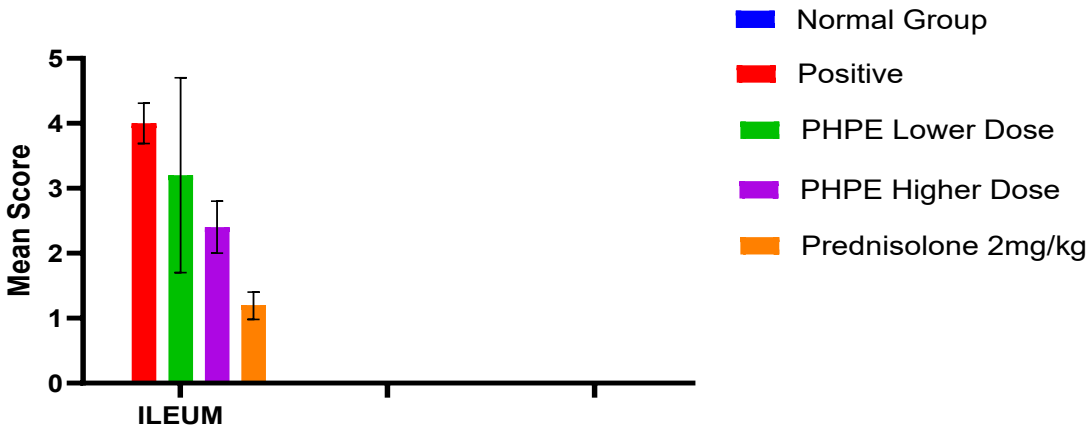
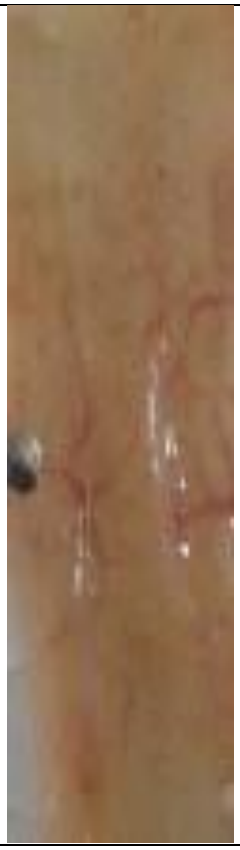


Fig. 3: Effect of extract treatment on macroscopic scoring of Ileum

All value are mean ± SEM of n=5 One way ANOVA followed by Tukey's post-test.

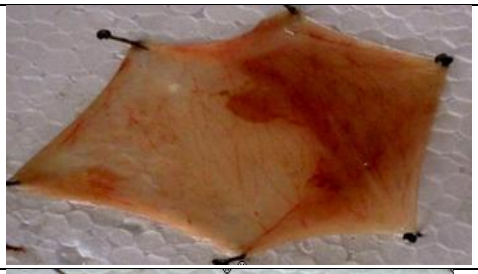
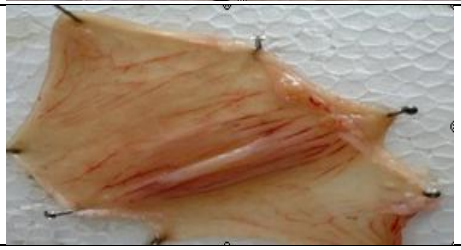
Significant difference between score of caecum of normal and positive control group was recorded (P<0.001). *P<0.05 Positive Control Vs Prednisolone (2 mg/kg), β P<0.001Positive Control Vs PHPE (Higher Dose) & Ns (Not Significant) Positive Control Vs PHPE (Lower Dose)

Macroscopic evaluation & Photographs of Indomethacin induced Model in Albino Wistar rats [Colon Part]

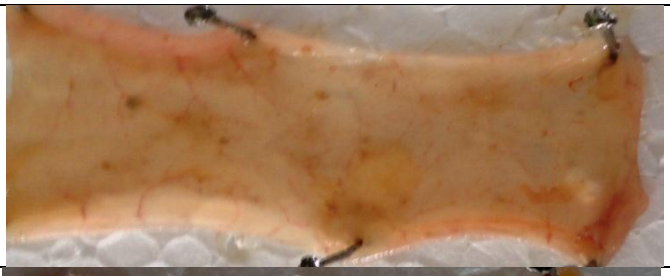
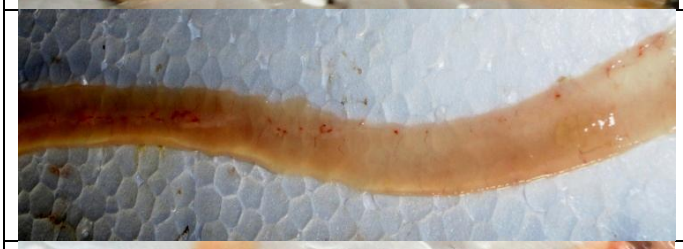
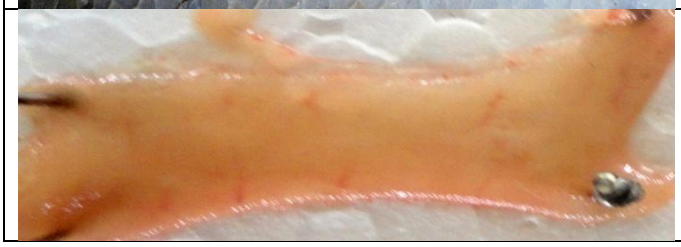
				
Figure-1	**Figure-2**	**Figure-3**	**Figure-4**	**Figure-5**
Normal Group	**Positive Control**	**PHPE Lower Dose**	**PHPE Higher Dose**	**Prednisolone 2mg/kg**

Macroscopic evaluation & Photographs of Indomethacin induced Model in Albino Wistar rats [Caecum Part]

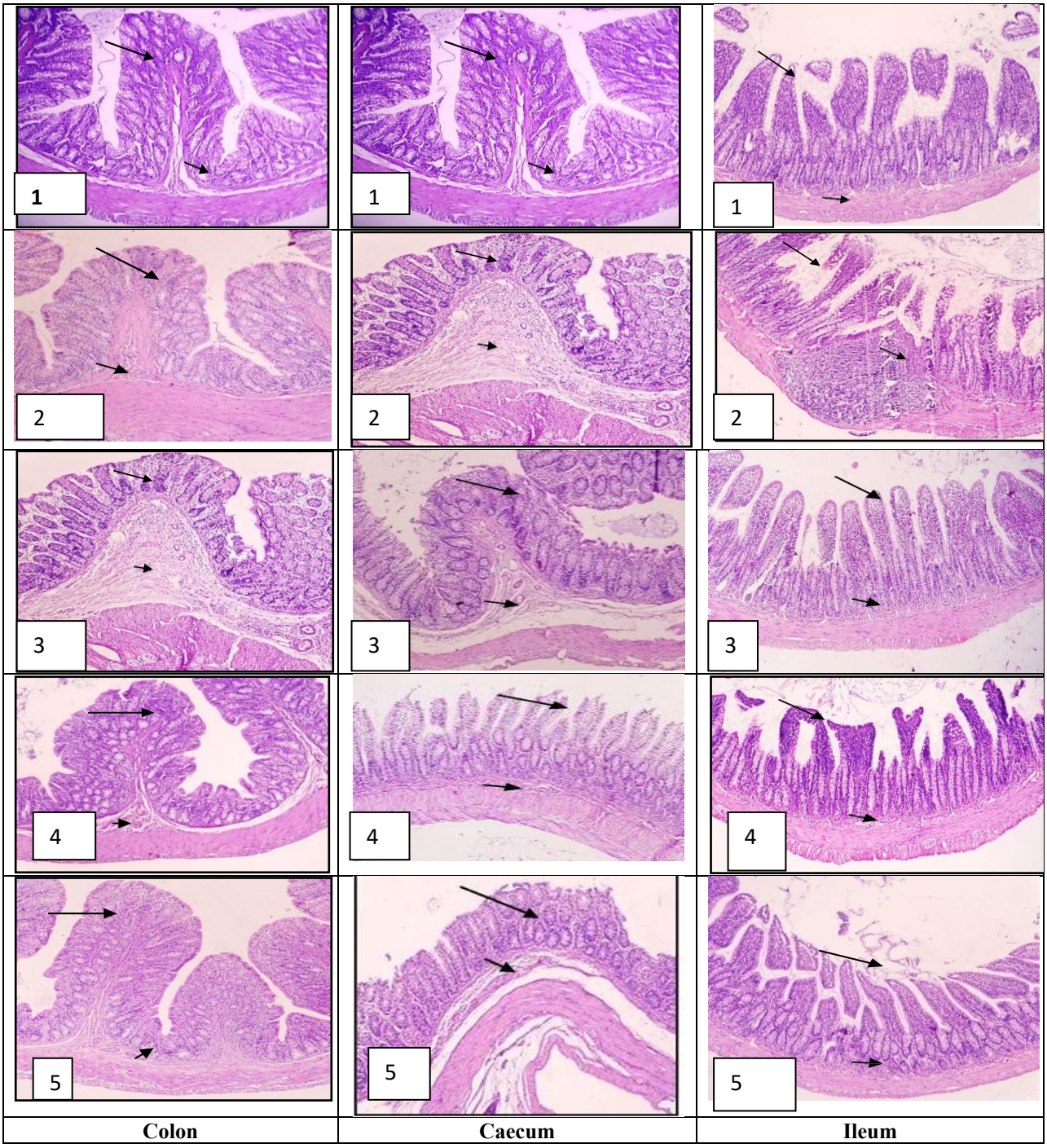
	**Figure -1**	**Normal Group**
	**Figure -2**	**Positive Control**
	**Figure -3**	**PHPE Lower Dose 500mg/kg**

	**Figure -4**	**PHPE Higher Dose 1000 mg/kg**
	**Figure -5**	**Prednisolone 2mg/kg**

Macroscopic evaluation & Photographs of Indomethacin induced Model in Albino Wistar rats [Ileum Part]

	**Figure -1**	**Normal Group**
	**Figure -2**	**Positive Control**
	**Figure -3**	**PHPE Lower Dose**
	**Figure -4**	**PHPE Higher Dose**
	**Figure -5**	**Prednisolone 2mg/kg**

Microscopic assessment [Histopathology] report of Indomethacin induced Model in Albino Wistar rats. (Colon, Caecum & Ielum)



Colon Section

1. Section studied from the colon shows intact mucosa lined by glandular epithelium
2. Section studied from shows ulcerated mucosa with areas of necrosis
3. Section studied from the colon shows intact mucosa lined by glandular epithelium
4. Section studied from the colon shows intact mucosa lined by glandular epithelium
5. Section studied from the colon shows intact mucosa lined by glandular epithelium

Caecum Section

1. Section studied of the caecum shows intact mucosa lined by glandular epithelium.
2. Section studied from caecum shows focal ulceration and areas of necrosis within the mucosa

3. Section shows from the caecum shows intact mucosa lined by glandular epithelium
4. Section shows intact mucosa lined by glandular epithelium
5. Section studied intact mucosa lined by glandular epithelium

Ileum Section

1. Section studied from the ileum shows intact mucosa with villi lined by glandular epithelium.
2. Section studied from the ileum shows focal ulcerated mucosa with loss of villi.
3. Section studied from the ileum shows intact mucosa with villi and lined by glandular epithelium.
4. Section studied from the ileum shows intact mucosa with villi and lined by glandular epithelium.
5. Section studied from the ileum shows intact mucosa with villi and lined by glandular epithelium

Effect of Polyherbal plant extracts treatment on MPO & LPO Values.

Table 3: Effect of Polyherbal plant extracts treatment on MPO & LPO Values

Groups	Treatment Groups	MPO activity (U/g) ± S.E.M	LPO Activity (µmol/g) S.E.M
1	**Normal**	2.16 ±0.34	0.16 ±0.03
2	**Positive Control**	12.66 ±0.72*	0.70 ±0.05*
3	**PHPE Lower Dose**	8.34 ±0.84 [a]	0.47 ±0.07[b]
4	**PHPE Higher Dose**	4.00 ±1.13 [b]	0.3 ±0.07[c]
5	**Prednisolone (2mg/kg)**	1.68 ±0.21[c]	0.14 ±0.02[Ns]

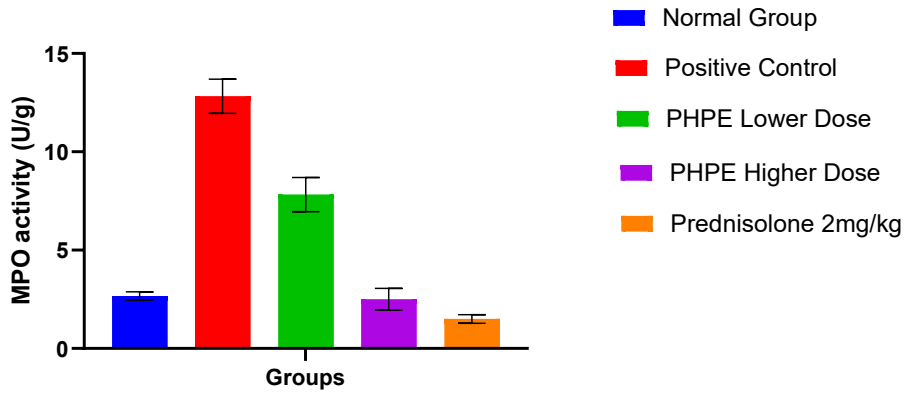


Figure 4: Effect of Polyherbal plant extracts treatment on MPO Values

Effect of PHPE on MPO: The myeloperoxidae assay showed significantly increase level of MPO activity Normal to Positive Control group *P<0.001, When compare to PHPE (Lower Dose) Vs Positive Control group b P<0.01, When compare with Positive control Vs PHPE (Higher Dose) c P<0.001, When compare with Positive control Vs Prednisolone (2mg/kg) c P<0.001.

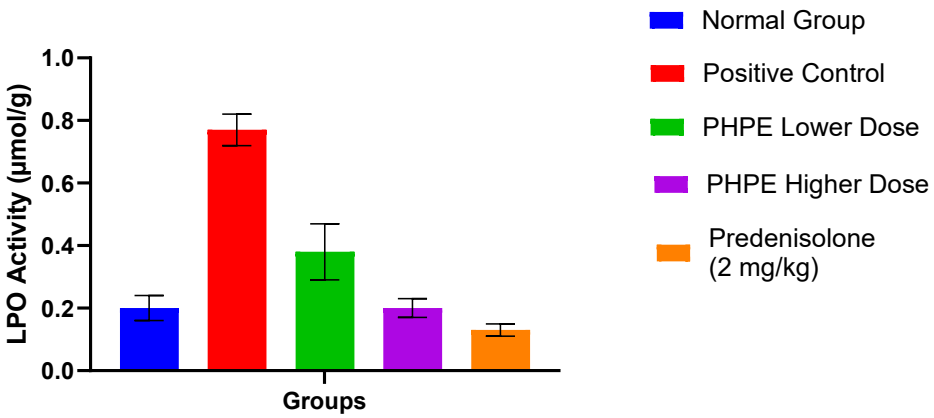


Figure 5: Effect of Polyherbal plant extracts treatment on LPO Values

Effect of PHPE on LPO: The Lipid Peroxidase assay showed significantly increase level of LPO activity Normal to Positive Control group *P<0.001 When compare to PHPE (Lower Dose) Vs Positive Control group b P<0.01, When compare with Positive control Vs PHPE (Higher Dose) c P<0.001, When compare with Positive control Vs Prednisolone (2mg/kg) c P<0.001.

CONCLUSION

These phytochemicals in the seeds of Centratherum anthelminticum and the roots of Hemidesmus indicus have been shown to have a wide range of effects, including cell proliferation, anti-inflammatory properties, and antioxidant stress reduction. Based on the results of the current study, it is concluded that methanolic extracts of the roots of Hemidesmus indicus and Centratherum anthelminticum have significant antioxidant and free radical scavenging capabilities and that the extract offers strong protection against Indomethacin-induced Enterocolitis.

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