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Abstract	In these present study, two different series of experiments such as evaluation for toxicity and therapeutic of effectiveness of Aesculus hippocastanum co-administered low viscosity of sodium alginate has been carried out by using rat model (Acetic acid-induced ulcerative colitis). Acute and subacute toxicities were performed according to OECD guideline-423 and 407, respectively. In vitro antioxidant study for the combination therapy was studied. Histopathological examinations were carried out to determined organ level toxicity on long-term use of such combination. The results for In vitro antioxidant study suggested the free radical scavenging activity of the combination therapy. There were no behavioral changes or any morbidity and mortality were observed during the oral acute toxicity study food intake, water intake, and body weight variation in all the group of animals were within the similar pattern that means no much changes has been observed. Among the animals between control tests and standard drug-treated groups. The result of histopathological data indicated that some changes were observed for hemoglobin content, red blood cell count, etc., in some test group which is negligible in comparison with control group. The microscopic feature of histopathological study for the different organs such as kidney, liver, heart, etc., indicated that some degeneration and necrosis were observed in all the test groups of animals. These alterations of histopathological changes may be due to the stress, infection, and administration of test compound in empty stomach. Further study is suggested for determination of appropriate dose and ratios (for combination) to reduce the long-term toxicity and to improve the therapeutic effectiveness for the benefit of the entire society.

Keywords	Aesculus hippocastanum - Low viscosity sodium alginate - Acetic acid antioxidant - Ulcerative colitis - Lipidperoxidase - Tumor Necrosis Factor (TNF-α)

Evaluation for Toxicity and Improved Therapeutic Effectiveness of Natural Polymer Co-administered Along with Venocin in Acetic Acid-Induced Colitis Using Rat Model

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Abstract In these present study, two different series of experiments such as evaluation for toxicity and therapeutic of effectiveness of Aesculus hippocastanum co-administered low viscosity of sodium alginate has been carried out by using rat model (Acetic acid-induced ulcerative colitis). Acute and subacute toxicities were performed according to OECD guideline-423 and 407, respectively. In vitro antioxidant study for the combination therapy was studied. Histopathological examinations were carried out to determined organ level toxicity on long-term use of such combination. The results for In vitro antioxidant study suggested the free radical scavenging activity of the combination therapy. There were no behavioral changes or any morbidity and mortality were observed during the oral acute toxicity study food intake, water intake, and body weight variation in all the group of animals were within the similar pattern that means no much changes has been observed. Among the animals between control tests and standard drug-treated groups. The result of histopathological data indicated that some changes were observed for hemoglobin content, red blood cell count, etc., in some test group which is negligible in comparison with control group. The microscopic feature of histopathological study for the different organs such as kidney, liver, heart, etc., indicated that some degeneration and necrosis were observed in all the test groups of animals. These alterations of histopathological changes may be due to the stress, infection, and administration of test compound in empty stomach. Further study is suggested for determination of appropriate dose and ratios (for combination) to reduce the long-term toxicity and to improve the therapeutic effectiveness for the benefit of the entire society.

Keywords Aesculus hippocastanum · Low viscosity sodium alginate · Acetic acid antioxidant · Ulcerative colitis · Lipidperoxidase · Tumor Necrosis Factor (TNF-α)

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1 Introduction

Inflammatory bowel disease (IBD) is an intestinal disorder leads to inflamed and ulcerative intestine. It includes Crohn's disease and ulcerative colitis. Ulcerative colitis affected rectal and colonic mucosa, (Carter et al. 2004.) This is including Crohn's disease and ulcerative colitis which is an idiopathic inflammatory bowel disease of the rectal and colonic mucosa (Morris et al. 1989). It is characterized by colonic inflammation, resulting most probably from the infiltration of polymorphonuclear cells, lymphocytes, monocytes, and plasma cells, accompanied by the overproduction of oxygen free radicals, ultimately leading to mucosal alteration and ulceration (Almenier et al. 2012). Ulcerative colitis is a chronic disease cause disturbance in homeostasis in the gastrointestinal tract and intestinal inflammation (Baumgart and Carding 2007). It is also affecting the mucosal layer of the distal colon and rectum. The main symptoms of ulcerative colitis include diarrhea, abdominal cramps, and recurrent blood in the stools caused by mucosal ulcers. Ulcerative colitis about 50 lakh people have affected by inflammatory bowel disease across the world and India. Annually, 12 lakh cases of IBD have been reported in India but unfortunately only few people are aware about the disease (Lennard-Jones 1989). The recent pharmacological therapy for patients with ulcerative colitis includes nonselective anti-inflammatory drugs and corticosteroids or immunosuppressants, as well as anti-TNF-α agents (Cho et al. 2007). Several abovementioned drugs are used for the treatment of ulcerative colitis but they possess several adverse effects. Hence herbal remedies came into existence as an alternative therapy to overcome the disadvantages of such drug (Kane et al. 2003). These drugs are used to maintain continuing long-term remission, reduction of abnormal colonic inflammation, and control of clinical symptoms, such as diarrhea, rectal bleeding, and abdominal pain (Mowat et al. 2011). Though, the continuous use of these medications can cause serious side effects to patients. Thus, a great effort has been made to develop new drugs to treat ulcerative colitis.

It has been reported that herbal drugs are the best alternative for the treatment of ulcerative colitis. Herbal drugs are safe in comparison to other existing drugs, these can be used to prevent long-term remission, reduction of colonic inflammation, controlling clinical symptoms including rectal bleeding, diarrhea, and abdominal pain. Therefore, to avoid serious adverse effects of allopathic drugs, greater efforts have been paying to develop new drugs to treat ulcerative colitis. The natural herbal drugs Aesculus hippocastanum is used by several researchers for the effective treatment of deep vein thrombosis and other venous disorders which is generally seen in ulcerative colitis by considering the therapeutic effectiveness of Aesculus hippocastanum. Hence we have decided to use Aesculus hippocastanum as a test substance to evaluate its potency. The Sodium alginate is well known as biocompatible, degradable, and nontoxic. It forms a gel without the need of heat. They are also widely used as protective reparative effects. So, in this research we have proposed to administer the natural polymer sodium alginate alone and in combination

with Aesculus hippocastanum in their appropriate ratio to determine the toxicity and therapeutic effectiveness against Acetic acid-induced ulcerative colitis in rat model.

2 Materials and Methods

2.1 Drug and Chemical Reagents

Aesculus hippocastanum was received as a gift sample from SUNPURE Pvt. Ltd New Delhi (India). Sodium Alginate was obtained from SD FINE Chem Ltd. Mumbai (India). Glacial acetic acid (AA) 99.8% was purchased from LOBA Chemie Pvt. Ltd. Mumbai (India). Sulfasalazine was procured from WALLACE Pharmaceuticals Pvt. Ltd. Ponda, Goa Maharashtra, (India). The RayBio® Enzyme-linked immunosorbent assay (ELISA) kits for rat TNF-α was obtained from Norcross USA. Lignocaine HCl Gel was purchased from ALVES Healthcare Pvt. Ltd. Mumbai, (India). All other chemicals used were of highest analytical grade commercially available.

2.2 Experimental Animals

Healthy adult Male Albino Wistar rats weighing about 180–200 gm were obtained from the Animal House Facility of Columbia Institute of Pharmacy, Raipur, Chhattisgarh, (India) having certificate number CIP/IAEC/2017/102 and Regd. No. 1321/PO/ReBi/S/10/CPCSEA. The animals were kept maintained under controlled environmental conditions with temperature (23 ± 2 °C), relative humidity (40–50%), and 12/12 h light/dark cycle with unlimited access to standard pelleted diet (chow, food) and water ad libitum, as per CPCSEA guideline. The animals were acclimatized to laboratory conditions for at least seven days before initiation of the experiment.

2.3 Experimental Design

The animals were randomly separated into five groups each containing six animals. Group I (Negative control) was pretreated with vehicle every 12 h, per oral; Group II (Toxic control) acetic acid, 2 ml; Group III (Test group-1) received AesculusHippocastanum 5 mg/kg oral; Group IV (Test group-2) received LVA 5gm/kg intrarectally; Group V (Test group-3) received 5 mg/kg mixture of Aesculus hippocastanum and LVA, intrarectally; Group VI (Reference group) was treated with Sulfasalazine 24 h before acetic acid instillation and for the subsequent five days.

2.4 Acute Toxicity

The acute toxicity was evaluated as per OECD guideline-423. The animals were randomly divided into five groups. They were received a dose of low viscosity sodium alginate along with Aesculus hippocastanum in Wistar rat of 50 mg/kg, body weight orally administered by using oral gavages after short fasting period. The general behavior of the animals was continuously monitored for 30 min, 1, 2, and 3 h after dosing, periodically during the first 24 h and the same treatment was followed for seven days.

2.5 Induction of Colitis

Inductions of ulcerative colitis were used according to the method of Ghasemi-Pirbaluti et al. (2017), with slight modification. The animals have fasted overnight with free access to water. The animals were light anesthetized with halothane. The inducing agent; acetic acid (2 ml, 1%, v/v) was instilled into the anus verge by inserting a medical grade polyurethane tube with 2 mm diameter through the rectum into the colon to a distance of 8 cm. The tube was kept in vertical position during instillation and after instillation to avoid leakage of acetic acid solution. Following the enema, After that, animals were kept in cages with continuous supply of feed and water till 8th day. Halothane was used to anesthetize animals and biochemical estimation was performed by collecting blood by retro-orbital puncture for biochemical estimation. The animals were again anaesthetized by using excess halothane and sacrificed by cervical dislocation. The abdominal portions were cut opened and colon was dissected out. Colon was flushed gently with saline and weighed. It was used for macroscopic scoring and histopathological estimations.

2.6 Hematological Study

The blood was collected with EDTA anticoagulant through retro-orbital puncture for biochemical estimation. The evaluated blood parameters were red blood cell count, blood hemoglobin concentration, basophil, eosinophil and neutrophil granulocytes, lymphocytes, and monocytes, hematocrit, mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin concentration (MCHC), white blood cell, and platelet counts (Byelinska et al. 2018).

2.7 Antioxidant Activity Lipid Peroxidase/Malonaldehyde (LPO/MDA)

A colonic tissue sample was homogenized in potassium phosphate buffer (50 mMol/L, pH 7.4, 1 g tissue/5 mL buffer). The total amount of protein in each sample was measured using the Bradford method. The tissue homogenate (10% w/v) was prepared in 0.15 M Tris-HCl buffer (PH 7.4). Then to it 0.2 ml of 8.1% sodium dodecyl sulphate (SDS) + 1.5 ml 20% acetic acid + 1.5 ml 8% Thiobarbutric acid (TBA) were added and volume was made up to 4 ml with distilled water. The above solution was subjected to heat on water bath for 60 min using glass ball as condenser. Then the solution allowed cooling and volume was made up to 5 ml. Then 5 ml of butanol: pyridine (15:1) was added and vortexed for a period of 2 min followed by centrifuge at 3000 rpm for 10 min. The upper organic layer was taken and measured optical density was measured at 532 nm. The absorbance was considered as total malondialdehyde (MDA) formed (Bose et al. 1989; Hagar et al. 2007; Alam et al. 2013).

2.8 Measurement of TNF-α

Colon was removed and homogenized in PBS then the amount of protein in each sample was measured via the Bradford method. The results were expressed in pg of cytokine/mg of protein. Assessment of cytokines (TNF- α) was carried out using ELISA kit; in clonic tissue, strips were minced with scissors for 15 s, suspended in 2 ml of 10 mm PBS (7.4 pH) and incubated in a shaking water bath 37 °C for 20 min. The sample was centrifuged and the supernatants were kept at −70 °C. The TNF-α assay using ELISA kit was performed (Wallace et al. 1989; Bose et al. 1989).

2.9 Histopathological Evaluation

The samples of highest macroscopic damage were selected from the sections of rat colon tissue. A two cm portion of the colonic tissue specimen from each animal was removed and fixed in 10% formalin solution then cut into 5 μm thickness, stained using hematoxylin eosin for the histopathological examination. They were made using a rotary microtome, 5 μm thickness sections were cut from the tissue samples embedded in paraffin and placed on standard glass slides. The paraffin was melted with a period of approx 12 h in an incubator at 58 °C. The samples were then stained with haematoxylene and eosin (H&E) according to the protocol. Qualitative analyses were performed on 400× magnified images.

Table 1 Observation table of animals behavioral

Test	Gender	Control	Control Reversal	Test-1	Test-2	Test-3	Test-3 reversal
Tremor	M	–	–	–	–	–	–
	F	–	–	–	–	–	–
Convulsion	M	–	–	–	–	–	–
	F	–	–	–	–	–	–
Salivation	M	–	–	–	–	–	–
	F	–	–	–	–	–	–
Diarrhea	M	–	–	–	–	–	–
	F	–	–	–	–	–	–
Sleep	M	–	–	–	–	–	–
	F	–	–	–	–	–	–

3 Result

3.1 Behavioral Changes

The results of oral acute toxicity study indicated that behavioral changes were no mortality and morbidity observed in animals through the 3-days period following single oral administration at all selected dose levels of the low viscosity sodium alginate along with Aesculus hippocastanum (Table 1).

3.2 Body Weight Loss

The result of body weight in different groups of animals reveled that there were no much changes have been observed (Table 2 and Fig. 1).

3.3 Hematological Study

See Tables 3 and 4.

3.4 MDA Activity

See Table 5 and Fig. 2.

Table 2 Effect of Low viscosity sodium alginate along with Aesculus hippocastanum on body weight in Albino Wistar Rats

Time period	Gender	Control	Control reversal	Test-1	Test-2	Test-3	Test-3 reversal
0 Days	M	182.4 ± 2.97	182.6 ± 2.57	182.4 ± 2.97	182.5 ± 2.51	182.6 ± 2.57	182.8 ± 2.98
	F	181.2 ± 1.43	181.5 ± 1.33	181.6 ± 1.54	181.5 ± 1.33	181.4 ± 1.25	181.4 ± 1.32
7 Days	M	183.6 ± 2.13	183.8 ± 2.15	183.5 ± 2.11	183.6 ± 2.12	183.7 ± 2.15	183.8 ± 2.18
	F	182.1 ± 2.12	182.3 ± 2.13	182.5 ± 2.11	182.5 ± 2.13	182.6 ± 2.11	182.9 ± 3.1
14 Days	M	184.5 ± 3.15	184.8 ± 3.16	184.2 ± 3.12	184.6 ± 3.15	183.8 ± 3.18	183.9 ± 3.19
	F	183.2 ± 3.03	183.5 ± 3.05	183.2 ± 3.04	183.3 ± 3.06	183.5 ± 3.07	183.7 ± 3.09
21 Days	M	185.3 ± 2.92	185.4 ± 2.94	185.1 ± 2.93	185.2 ± 2.94	185.5 ± 2.96	185.7 ± 2.97
	F	183.4 ± 2.15	183.6 ± 2.16	183.1 ± 2.07	183.8 ± 2.16	183.6 ± 2.12	185.8 ± 2.99
28 Days	M	185.8 ± 2.98	185.9 ± 2.96	185.5 ± 2.96	185.4 ± 2.94	185.6 ± 2.97	185.8 ± 2.99
	F	185.2 ± 2.94	185.5 ± 2.96	185.4 ± 2.29	185.2 ± 2.94	185.7 ± 2.97	185.9 ± 2.99

Mean ± SEM (n = 6)

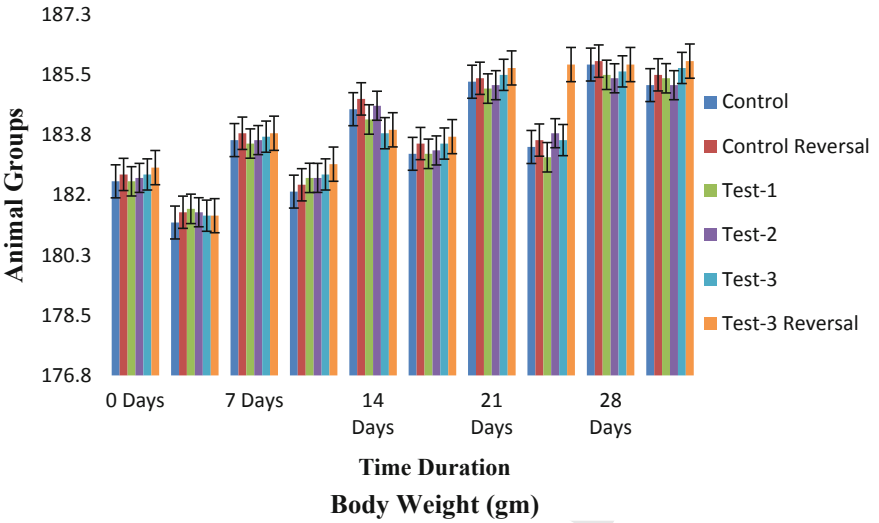

Fig. 1 Graphical representation of animals mean body weight during dosing, number of animals per group n = 10, each group (Five—Male and Five—Female). All value is reported as mean ± SEM (n = 6)

Table 3 Hematological data of various groups of male animals

S. no.	Parameters	Control group	Test-1 group	Test-2 group	Test-3 group
1.	Hb (gm%)	16.48 ± 0.298	16.46 ± 0.102	16.26 ± 0.102	16.2 ± 0.2
2.	WBC (cmm)	2560 ± 40	3420 ± 152.9	4360 ± 112.2	4480 ± 106.77
3.	Neu (%)	42.4 ± 0.244	40.4 ± 0.812	43.8 ± 0.969	44 ± 00
4.	Lym (%)	52 ± 0.004*	53.8 ± 0.969	47.8 ± 0.734	47.2 ± 0.244
5.	Eos (%)	4.2 ± 0.374	5 ± 0.316	4.6 ± 0.244	4.4 ± 0.509
6.	Mon (%)	01 ± 00	01 ± 00	01 ± 00	01 ± 00
7.	Bas (%)	00 ± 00	00 ± 00	00 ± 00	00 ± 00
8.	RBC (%)	7.61 ± 0.002*	7.53 ± 0.046	7.54 ± 0.011	7.53 ± 0.009
9.	Platelet (%)	2.75 ± 0.003*	2.91 ± 0.019	2.94 ± 0.007	2.95 ± 0.013
10.	MPV	9.62 ± 0.058	9.46 ± 0.097	9.4 ± 0.054	9.5 ± 0.004*
11.	PCV	33.38 ± 0.165	33.40 ± 0.329	33.42 ± 0.631	33.46 ± 0.082
12.	MCV	51 ± 0.196	52.69 ± 0.087	52.72 ± 0.12	52.75 ± 0.01
13.	MCHb (Pictograms)	19.72 ± 0.009	19.68 ± 0.026	19.92 ± 0.002*	19.68 ± 0.058
14.	MCHb (mg/dl)	36.47 ± 0.006	37.35 ± 0.011	37.63 ± 0.022	37.66 ± 0.009
15.	RCDW (%)	15.22 ± 0.037	15.28 ± 0.086	15.5 ± 0.094	15.6 ± 0.005*

Mean ± SEM (n = 5), P value = < 0.005(*)

Table 4 Hematological data of various groups of female animals

S. no.	Parameters	Control group	Test-1 group	Test-2 group	Test-3 group
1.	Hb (gm%)	15.48 ± 0.298	14.46 ± 0.102*	14.26 ± 0.102	14.2 ± 0.2
2.	WBC (cmm)	2350 ± 41	2352 ± 42.3	2355 ± 42.2	2355 ± 43.7
3.	Neu (%)	42.4 ± 0.24	40.4 ± 0.23	43.8 ± 0.29	44 ± 00
4.	Lym (%)	51 ± 0.70	51.4 ± 0.69	52.4 ± 0.73	52.6 ± 0.74
5.	Eos (%)	4.2 ± 0.34	4.5 ± 0.36	4.6 ± 0.34	4.6 ± 0.39
6.	Mon (%)	01 ± 00	01 ± 00	01 ± 00	01 ± 00
7.	Bas (%)	00 ± 00	00 ± 00	00 ± 00	00 ± 00
8.	RBC (%)	7.61 ± 0.002	7.63 ± 0.046	6. ± 0.011	7.60 ± 0.009*
9.	Platelet (%)	3.75 ± 0.003	3.91 ± 0.019*	2.74 ± 0.007	3.14 ± 0.013
10.	MPV	9.62 ± 0.058	9.46 ± 0.097	10.3 ± 0.054	10.6 ± 0.050*
11.	PCV	47.38 ± 0.165	33.84 ± 0.329	38.22 ± 0.631	42.46 ± 0.082
12.	MCV	55.41 ± 0.196	51.22 ± 0.087	52.724 ± 0.12	49.938 ± 0.01
13.	MCHb (Pictograms)	19.72 ± 0.009	20.68 ± 0.026	19.92 ± 0.02*	18.68 ± 0.058
14.	MCHb (mg/dl)	35.47 ± 0.006	40.35 ± 0.011	37.73 ± 0.022	37.66 ± 0.009
15.	RCDW (%)	15.72 ± 0.037	16.28 ± 0.086*	16.5 ± 0.094*	16.36 ± 0.05*

Table 5 Serum MDA levels in different groups of animals

S. no.	Groups	MDA level
1	Control	8.22 ± 0.509
2	Test-1	5.23 ± 0.152
3	Test-2	4.57 ± 0.052
4	Standard	4.78 ± 0.072

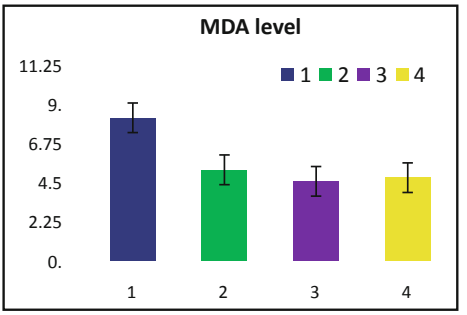

Fig. 2 Graph showing the level of serum MDA in homogenized colon tissue of different group of animals

Table 6 TNF-α level in different groups

S. No.	Groups	TNF-α level
1	Control	5.53 ± 0.672
2	Test-1	4.84 ± 0.252
3	Test-2	4.59 ± 0.239
4	Standard	5.13 ± 0.584

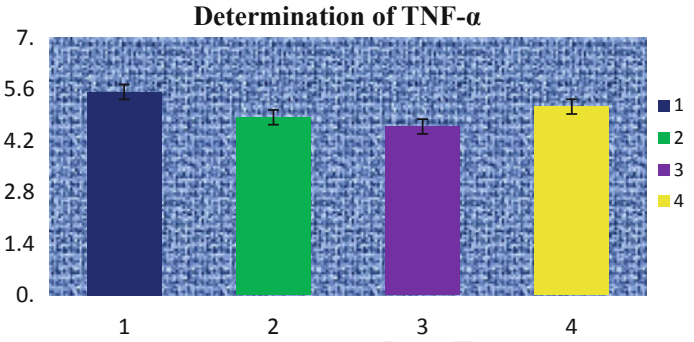

Fig. 3 Graph showing the level of TNF-α in homogenized colon tissue of different group of animals

3.5 TNF-α Activity

See Table 6 and Fig. 3.

3.6 Histopathology

See Fig. 4.

4 Discussion

The modern pharmaceutical research is concerned with all aspects of identifying new chemical substances with new modes of action. In particular, the economics of treatment linked to drug dosage has led compound to new drug development technologies. As a result, treatments are now becoming more reasonable for wide sections of society, including the financially challenged. Few marketed products are available for the effective treatment of IBD such as Sulfasalazine, Mesalazine, Balsalazide, Antileukotriene like Montelukast, etc. Natural polymers are evaluated for their wound-healing effect in case of IBD. Steroidal drugs such as prednisone,

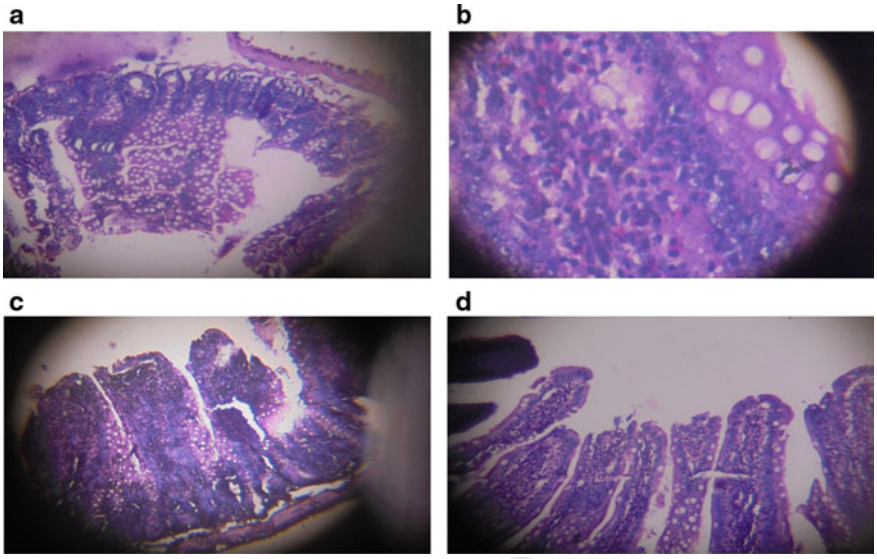

Fig. 4 **a** Control group. **b** Test group-1. **c** Test group-2. **d** Standard group

etc., on long-term use cause severe side effect like Cushing syndrome. Many herbal products possess anti-inflammatory, antioxidant activity has been evaluated for their anti-IBD effect. Venocin from Horse chestnut seed extract has been shown to have Antioxidant, Anti-inflammatory, Wound healing, and Supports circulation property.

Several abovementioned allopathic drugs are used for the treatment of ulcerative colitis but they possess several adverse effects. Hence herbal remedies came into existence as an alternative therapy to overcome the disadvantages of such drugs. Among the natural herbal drugs Aesculus hippocastanum is used by several researchers for the effective treatment of deep vein thrombosis and other venous disorder which is generally seen in ulcerative colitis by considering the therapeutic effectiveness of Aesculus hippocastanum. We have decided to use Aesculus hippocastanum as a test substance to evaluate its potency against acetic acid-induced ulcerative colitis using rat model. Further it has been evidenced from the literature that sodium alginate is a natural polymer used for various gastrointestinal disorders though sodium alginate is obtained from natural source and devoid of any side effects/adverse effects. The Sodium alginate is well known as biocompatible, degradable, and nontoxic. It forms a gel without the need of heat. They are also widely used as protective reparative effects. Though we have taken the combination of sodium alginate which is co-administered with Venocin, there is a need to evaluate the toxicity and safety of the combination.

Two series of studies have been carried out for the evaluation of toxicity and therapeutics effectiveness for the combination of Aesculus hippocastanum along with low viscosity sodium alginate at their predetermined dose.

The oral toxicity study has been carried out as per OECD-423. The results of the oral acute toxicity study indicated that there were no mortality and morbidity observed in animal of all the groups. The result of body weight in different groups of animals reveled that there were no much changes has been observed similarly, food and water consumption all the group of animals showing similar pattern of result during the entire course of experiment the effect of combination therapy on Hematological data of all the groups of animal when depicted in Table no. 15 and Fig no. 6–18. The hemoglobin content except control and control reversal group of animals reduced to a lesser extent whereas in case of animal of test-3 reversal group the value has been increased up to 17.3. The WBC count in all groups of animals has been increased. To some extent in comparison to control and control reversal group neutrophils lymphocytes and eosinophils value did not indicated much change in all groups of animals. RBC count has been decreased in Test-1, Test-2 as well as Test-3 reversal group whereas Test-3 group of animals having similar RBC count value in comparison to control reversal group platelet count and mean platelet value shown no much change in all group of animals. The value for packed cell volume has been reduced in Test-1, Test-2, and Test-3 reversal group of animals. The results for Mean corpuscular volume having similar pattern of results in all groups of animals. The results of mean corpuscular Hemoglobin content indicated that it has been increased in all the test groups of animal in comparison to control and control reversal group.

The antioxidant activity for the combination therapy has been determined by the estimation of MDA and TNF-α. The results for MDA have been depicted in table no 17, 18, and graphical represented in graph no. 20 and 21. The results indicated that Test compound showing decreased in concentration of MDA and TNF-α. Hence the combination therapy possesses free radical scavenging activity.

On the termination of experiment the animal was sacrificed as per CPCSEA guidelines and Subjected organ where isolated and subjected for histopathological examination to determine the toxicity of combination therapy at different organ level. The histopathological indicated that on long-term use of combination therapy some degeneration and necrosis have been observed in Test group of animals whereas no changes in microscopic features were pointed out in control and control reversal group of animals. These may be due to the excessive stress less food intake, etc. From the above finding it has been observed that the combination of Aesculus hippocastanum and Low viscosity sodium alginate at predetermined dosed possess very good free radical scavenging activity and anti-inflammatory activity and TNF-α inhibiting activity. To reduce the long-term use of organ level toxicity, further study is suggested to adjust the dose level and duration which produce better therapeutic effectiveness which in turn pave the way for the development of new drug.

5 Conclusion

The present study has been undertaken to establish the improved therapeutic effectiveness of Aesculus hippocastanum co-administered with LVA. From the previously published scientific data, Aesculus hippocastanum is used for the

treatment of various venous disorders such as deep vein thrombosis, etc., and Sodium alginate is a natural polymer having good mucosal protective activity. Hence, the present study has been proposed to determine the toxicity and synergistic effect of both the drugs with their appropriate ratios. The study has been carried out using Wistar rats and acetic acid was used as inducing agent for ulcerative colitis. Various In vitro studies such as Malondialdehyde (MDA) carried out and the result revealed that the combination possess good level of antioxidant activity. Hematological and biochemical findings also support our hypothesis but long-term administration of combinations (AesculusHippocastanum along with low viscosity sodium alginate) alters the mucosal integrity which has been observed from histopathological findings of some vital organs. So, our findings suggest that further detailed study is required to establish the exact mechanism of mucosal degeneration which in turn reduces the long-term organ level toxicity that will help the researchers to decide for further new drug development for the benefit of the society.

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