



# Therapeutic Effect of *Aloe vera* on Croton Oil Induced Hemorrhoids

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## Abstract

Hemorrhoids are vascular structures in the anal canal with swollen veins in the lowest part of the rectum/anus. Till date, there is no specific treatment available for different grades of hemorrhoids. However, it is evident that lesser invasive options are to have opted for at the earliest along with conservative measures such as analgesics and anti-inflammatory agents. The study evaluates the efficacy of gel obtained from *Aloe barbadensis* Miller in treating hemorrhoids in a croton oil-induced Wistar rat model. Animals were allocated into 5 groups. Group I and II served as the normal and negative control, Group III as standard, and Group IV and V received an oral route and a topical route respectively. Biochemical parameters such as TNF- $\alpha$  and IL-6 were estimated in each group after the therapy of 5 days. Quantitative estimation of edema was done by determining the Evans blue dye concentration in the plasma exudates in recto-anal tissue. The recto-anal coefficient was estimated as a direct measure of inflammation. Histopathological variations among the groups were analyzed. Statistical analysis of the data affirms the curative effect of aloe vera on Hemorrhoids. Results demonstrate that aloe gel is much more effective when compared to Pilex ointment which was used as standard ( $p < 0.05$ ). However, oral preparation was not found to be effective in mitigating the disease as that standard ( $p > 0.05$ ).

**Keywords:** *Aloe barbadensis* Miller, Anti-inflammatory, Evans Blue Dye, Hemorrhoids

## 1. Introduction

Hemorrhoids are vascular structures in the anal canal that help with stool control. Hemorrhoids are referred to as a disease when these veins become swollen or inflamed upon stress/pressure, which may further lead to bleeding, pain, and irritation during and after defecation in the initial stages. Progression of the disease can decrease the quality of life. Hemorrhoids are classified into two major types, viz., External and Internal Hemorrhoids based on the location of the swollen veins with reference to the dentate line. Due to the somatic nerve supply below the dentate line, the presence of pain-sensing areas innervates the pain in External hemorrhoids. Owing to the lesser pain-sensing areas in the visceral nerve fibers above the dentate line, the perception of pain is usually not innervated as it is well-rooted inside the abdomen<sup>1</sup>. Both Internal

and external hemorrhoids which do not prolapse or thrombose are most probable to heal themselves without any onset of symptoms or complications. The Goligher system of classification is done by four grades / Prolapse degree viz., Grade I and II indicating non-prolapsed cushion bleeding but getting reduced spontaneously without manual support, Grade III and IV indicating prolapsed anal cushion upon straining and it can only be retracted into the anal canal manually<sup>2</sup>. Worldwide, the overall prevalence of symptomatic Hemorrhoids in the general population is estimated at around 4.4%, and around 40 million in India according to the recent surveys. Incidence of the disease seems to appear between the age of 45 to 65 years irrespective of gender. Various techniques have been deployed to view the disease, which includes sigmoidoscopy in ruling out the rectal mass, and full colonoscopy that excludes

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other causes, of colon rectum, and bleeding. Till date, suitable non-invasive treatment available for different grades of hemorrhoids is much debatable. However, it is observed that lesser invasive options are to be opted for first along with certain conservative measures<sup>1</sup>. The eminent theory known as sliding anal canal lining theory suggests that fragmentation of supporting tissue of anal cushion steering towards the downward direction leading to hemorrhoidal disease. Other such theories that explain the risk for disease include lack of dietary fibers, loitering around the lavatory for a long time, protracted labor (straining with difficulty), inactive lifestyle, and family history<sup>3-5</sup>.

The enzymes notable for fragmentation of supporting tissues of anal cushion include matrix metalloproteinase (Zinc dependent proteinase) is capable of degrading extracellular proteins viz., collagen, elastin, and fibronectin. MMP-9 (matrix metalloproteinase) is overexpressed in hemorrhoids and is involved in the fragmentation of supporting tissue of the anal canal and also disrupts the elastic fibers<sup>6</sup>. Coagulation and fibrinolysis key enzymes like plasmin, thrombin, and other proteases, activate MMP-2/ MMP-9 leading to interruption of capillary bed and activation of angio-proliferative activity of TGF-  $\beta$ <sup>7</sup>. Doctors propose that several medications such as low dose local anesthetics, corticosteroids, keratolytic, and antiseptics can be applied topically which might be relieving for post-hemorrhoidectomy symptoms. At the same time, the chronic application is not advisable.

Traditionally, Aloe Vera has been deployed to treat skin-related problems like wounds, burns, and anti-inflammatory processes. Additionally, it has shown other therapeutic properties including antioxidant, anticancer, antihyperlipidemic, and antidiabetic<sup>8</sup>. It also shows pharmacological properties to provide antioxidant, wound healing, antifungal, antibacterial and immunomodulatory effects<sup>9</sup>. In recent times, it has been proposed that Aloe Vera cream has the potential for wound healing in post-hemorrhoidectomy patients<sup>10</sup>. *Aloe barbadensis* Miller (ABM) is the most widely used type of Aloe due to its high efficiency of leaves, robustness, and ease of pulping for drinks or gels for external use. The gel is found to have potent anti-inflammatory activity in various experimental diabetes models suggesting its use in various diseases

involving inflammation. *In vivo* studies reveal that topical application of aloe vera leads to fast wound healing<sup>11</sup>. ABM supports as a supplement owing to its natural origin and various beneficial activities, possessing several bioactive compounds in the internal gel of the leaf. The synergic effect of ABM is observed in combination with amino acids, vitamins, enzymes, lipids, carbs, etc.<sup>12</sup>.

In this current study, we intend to investigate the therapeutic potential of Aloe Vera gel and juice for hemorrhoid treatment by evaluating TNF $\alpha$ , IL-6, rectoanal coefficients (RAC), and Evans Blue dye (EB dye) estimates of plasma exudates.

## 2. Materials and Methods

### 2.1 Drugs and Chemicals

All chemicals including pyridine, diethyl ether, Evans Blue dye was purchased from Sisco Research laboratory (SRL), Chennai depot, India. Croton oil was purchased from CAMEO Chemicals, India. Pilex ointment was purchased from Himalaya drug company, Bangalore, India. All the chemicals purchased for the study were of analytical grade and from reputed suppliers.

### 2.2 Plant Material and Preparation

The leaves from *Aloe barbadensis* Miller commonly known as Aloe Vera were collected in the month of June 2021 from Chengalpattu district of Tamil Nadu. The outer leaf of ABM which appeared most fleshy and healthy among all the leaves in the plant was chosen for the study. The plant part was identified and authenticated by Plant Anatomy Research Centre (PARC), Medicinal plant research unit, Tambaram, Chennai. After collection of plant material, the dark yellow colored resin was allowed to drain off completely by placing the leaves upright for 1-2 hours. As the resin was drained off, the leaves were washed properly and the outer thick layers of the leaves were peeled off so as to expose the gel underneath. Further, the gel was scooped off using a sterilized spoon or knife. The eluted gel was placed in a sterilized and air-tight container until formulating into juice or gel.

For aloe juice to be given orally, 30mg of Aloe Vera gel was further run in a blender with 60ml water for

one minute and was transferred to a sterilized air-tight container and refrigerated until use. For Aloe Vera gel, the scooped gel was run in a blender without adding water or any other excipients and transferred to a different sterilized air-tight container, and refrigerated until use.

### 2.3 Experimental Procedure

Ethical clearance for the handling of animals was obtained from the Institutional Ethical Committee constituted for the purpose (CPCSEA NO: IAEC.4.2020). Adult male Wistar albino rats weighing  $179.5 \pm 34.31$ g and 2 months of age were used in the study. Six animals were used in each group. The rats were housed under standard experimental conditions of room temperature ( $22 \pm 3^\circ\text{C}$ ), 12-hour light and dark cycle, optimum humidity (50%), and 6 animals in a cage. The animals were fed with a standard diet of food pellets and water *ad libitum*. The animals acquired humane care as per the Committee for the Purpose of Control and Supervision of Experimental Animals (CPCSEA). The experiment was conducted as per the conditions suggested by the Institutional Ethical Committee. All animals were acclimatized for a week before the study.

### 2.4 Grouping and Drug Administration

30 animals were randomly assigned to 5 groups based on body weight (6 in each group), G-I to G-V. G-I served as normal control and received a standard amount of water and food for nourishment. G-II served as a negative control, G-III animals served as a positive control, received the standard drug, Pilex ointment, G-IV animals received 1ml aloe juice peroral and G-V received aloe gel, 0.5ml topically applied in the anal region. The assigned treatment was given to all the groups except the GI and GII once a day, for 5 days after inducing hemorrhoids.

### 2.5 Induction of Hemorrhoid

Hemorrhoids were induced with croton oil in all the groups except GI which served as normal control as previously reported<sup>13</sup>. Briefly, croton oil preparation containing pyridine, diethyl ether, deionized water, and 6% croton oil diethyl ether in the ratio of 4:5:1:10 was

prepared. Hemorrhoid was induced by applying soft cotton bud soaked in the preparation was inserted into the recto-anal region, 2cm from the anal opening after being fasted overnight. Soaked cotton bud was kept in the recto-anal region for 25 seconds. Animals were observed for the development of edema in the recto-anal region after 8 hours of croton oil application. To evaluate the plasma exudation in the recto-anal region quantitatively due to edema formation, animals were injected with EB dye through the tail vein. 0.5ml of 2% EB dye solution was administered to all the groups one hour prior to induction. Treatments for respective groups were commenced one day after induction of hemorrhoids. Treatment was continued for 5 days.

### 2.6 Evaluating the Effect of Aloe against Hemorrhoid

On the 6<sup>th</sup> day, animals were weighed and blood samples were collected from the retro-orbital sinus of all the animals for estimating the biochemical parameters, TNF- $\alpha$  and IL-6 which were markers of inflammation. Animals were fasted overnight and sacrificed after being euthanized and exsanguinations under deep anesthesia. Recto-anal tissue was isolated by an incision made in the anal region extending towards the rectum. Approximately 2 cm of tissue recto-anal tissue was isolated as shown in Figure 1. The isolated tissue was weighed for estimating the Recto-Anal Coefficient (RAC).

RAC was measured using the following formula<sup>13</sup>

$$\text{RAC} = \frac{\text{Weight of recto-anal tissue in mg}}{\text{Weight of animals in g}}$$

Further tissue was fixed in 10% formal saline for histopathological evaluation. EB dye present in the tissue was extracted by fixing the tissue in 1 ml formamide. The absorbance of the sample thus obtained was measured using a spectrophotometer at 620nm and was further quantified using the standard curve of EB dye<sup>14</sup>.

### 2.7 Histopathology

Slices of recto-anal tissue were fixed in Bouin's solution for 12 hours. Further embedded in paraffin wax and sectioned at  $5\mu\text{g}$  onto the glass slides. Sections were stained with alum hematoxylin and eosin after

dewaxing. Sections were microscopically examined and histopathological changes between the groups were evaluated and compared under 40X magnification.

## 2.8 Statistics

Measure of RAC, biochemical parameters such as TNF- $\alpha$ , IL-6 and Evans blue dye estimates in recto-anal tissue being the statistical parameters were used as mean  $\pm$  standard deviation.  $p \leq 0.05$  was considered to be statistically significant. Statistical significance between any two groups were compared using unpaired *t*-test with 5% level of significance. Overall, statistical significance between the groups was compared using one way analysis of variance (ANOVA). All analysis was carried out using GraphPad prism, version 8.4.3.686 with 95% confidence interval.

## 3. Results

Hemorrhoid was induced in all the animals except those in GI by applying croton oil preparation in the recto-anal region. Exposing the recto-anal region with cotton bud soaked in croton oil preparation for 25 seconds were found to be sufficient to induce hemorrhoid as the biochemical parameters such as IL-6, TNF- $\alpha$  and the RAC was found to be significantly higher in GII when compared to GI (Table 1).

Mean increment in RAC of GII was found to be  $2.24 \pm 0.183$  units when compared to GI which was statistically significant (1.91 vs. 4.51; 95% CI 1.836 to 2.652;  $p < 0.0001$ ). Mean difference in TNF- $\alpha$  between GI and GII was found to be  $103.5 \pm 2.581$  which was found to statistically significant (95% CI 97.73 to 109.2;  $p < 0.0001$ ). Induction of hemorrhoid can be interpreted from IL-6 measures too as the mean difference was found to be highly significant between GI and GII (68.58 vs. 133.1; 95% CI 60.78 to 68.26;  $p < 0.0001$ ). Similarly, EB dye concentration of GII was found to be significantly different when compared to normal control group (0.018 vs. 0.04;  $p < 0.0001$ ). Aloe Vera gel application (0.5ml) has maintained the RAC measure near to normal control group, but was statistically significant (1.91 vs. 2.77; 95% CI 0.0981 to 0.6799;  $p$

= 0.0138) (Figure 2). Similar results were observed in measure of TNF- $\alpha$  and IL-6. TNF- $\alpha$  measured in GV differed by  $6.810 \pm 2.076$  units from GI (95% CI 2.185 to 11.44;  $p = 0.0083$ ) (Figure 3). The mean difference in IL-6 between GI and GV was found to be  $6.033 \pm 2.160$  which was found to be significant ( $p = 0.0190$ ) (Figure 4). Aloe Vera gel application significantly influenced the extravasation of Evans Blue dye from the tissue in GV when compared to GII (0.04 vs. 0.022; 95% CI -0.020 to -0.0159;  $p < 0.0001$ ). Administration of Aloe Vera oral juice (GIV) showed significant results maintaining the outcome measures which were not as effective as standard Pilex therapy (GIII). Mean RAC of GIV was not statistically significant when compared to disease control group (GII) (4.155 vs. 4.080; 95% CI -0.450 to 0.300;  $p = 0.665$ ) (Figure 2). However, the mean difference of TNF- $\alpha$  measured between GII and GIV was statistically significant (mean difference:  $6.967 \pm 2.260$ ; 95% CI -12 to -1.932;  $p = 0.0116$ ) (Figure 3). Similar results were depicted from IL-6 measures between GII and GIV (133.1 vs. 125.0; 95% CI -13.84 to -2.325;  $p = 0.0107$ ) (Figure 4). Extravasation of EB dye from recto-anal tissue showed statistically significant difference between GII and GIV (mean difference:  $0.004 \pm 0.0011$ ; 95% CI -0.0065 to -0.0014;  $p = 0.0061$ ) (Figure 5).

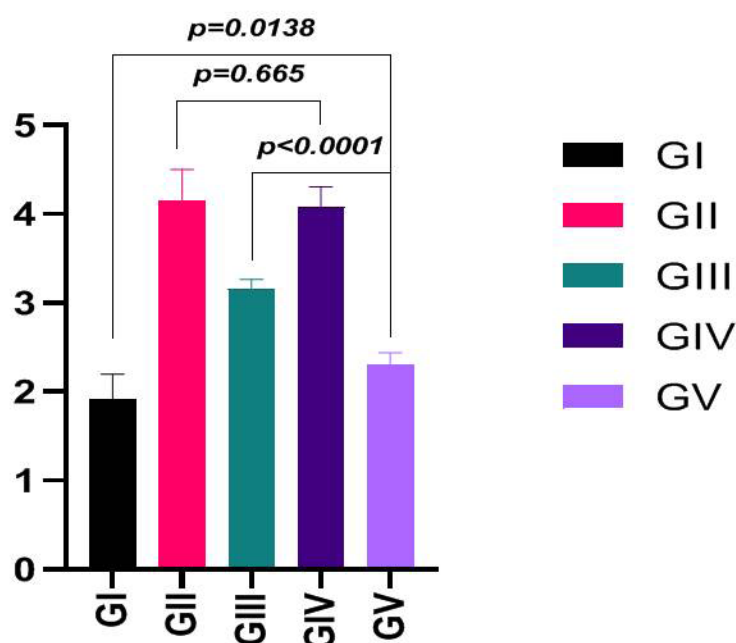


**Figure 1.** Recto-anal tissue isolated from hemorrhoid induced rat measuring 2cm.

**Table 1.** Efficacy outcomes measured after 5 days of treatment.

GROUP	RAC (mg/g)	TNF- $\alpha$ (pg/ml)	IL-6 (pg/ml)	EB DYE CONCENTRATION (mg/ml)
GI	1.911 $\pm$ 0.287	49.173 $\pm$ 3.381	68.583 $\pm$ 2.409	0.018 $\pm$ 0.002
GII	4.51 $\pm$ 0.847	152.65 $\pm$ 5.342	133.1 $\pm$ 3.332	0.04 $\pm$ 0.002
GIII	3.15 $\pm$ 0.070	67.916 $\pm$ 5.047	80.1 $\pm$ 1.694	0.025 $\pm$ 0.001
GIV	4.155 $\pm$ 0.345	145.683 $\pm$ 3.190	125.016 $\pm$ 2.158	0.036 $\pm$ 0.002
GV	2.3 $\pm$ 0.141	55.983 $\pm$ 3.798	74.616 $\pm$ 4.710	0.022 $\pm$ 0.001

Data are shown as mean  $\pm$  standard deviation. One way analysis of variance (ANOVA) was used to determine the overall significance. Any two groups were compared using unpaired *t*-test.

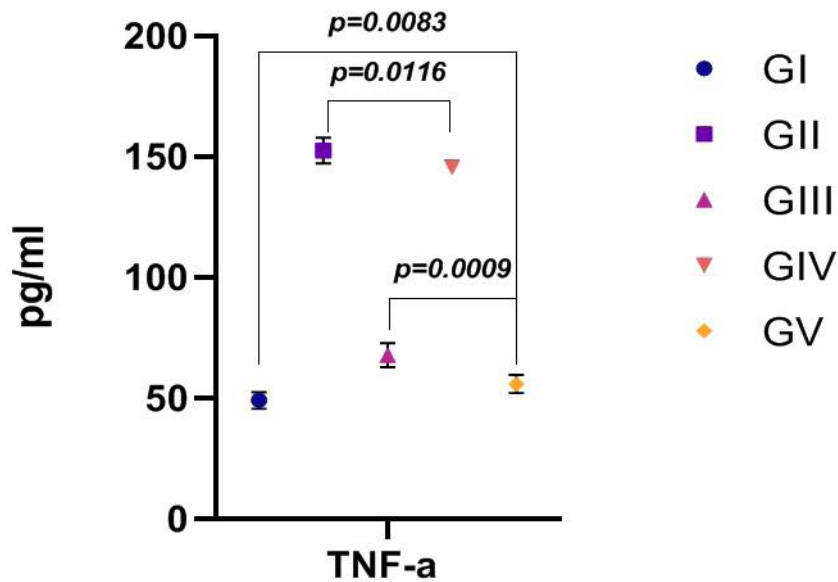


**Figure 2.** Effect of Aloe Vera (oral and topical administration) on RAC. X axis includes Groups I to V, whereas Y axis included Recto anal Coefficient (mg/g); GI represents control group, GII represents Negative control, GIII. represents cystone Standard group, Group IV represents oral route, and Group V represents topical route. Values are expressed as mean  $\pm$  SEM. Mean values are statistically compared using one way analysis of variance (ANOVA). Any two groups were compared using unpaired *t*-test.  $P < 0.05$  was considered to be statistically significant.

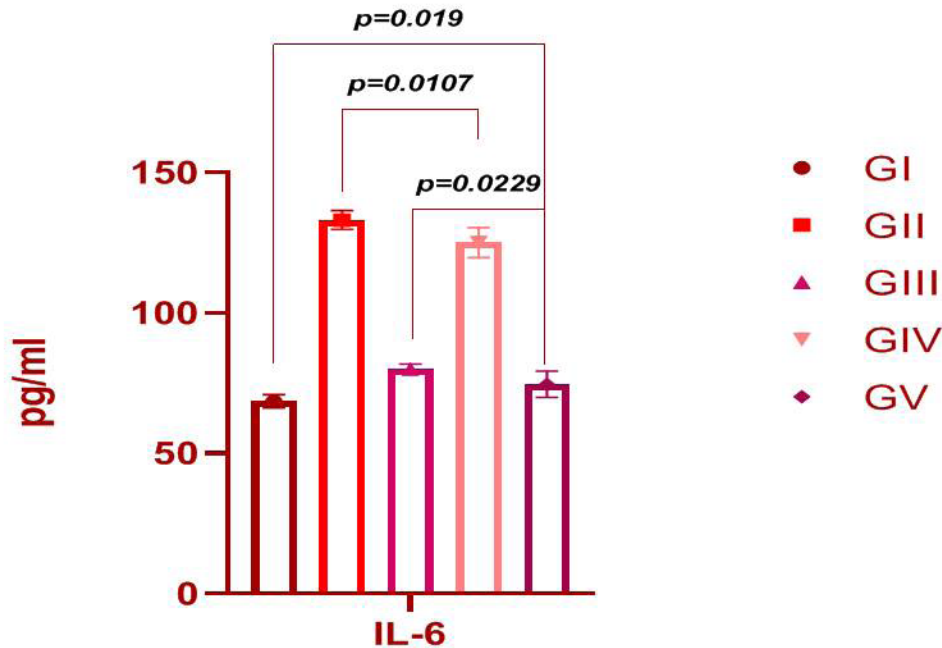
## 4. Discussion

Therapeutic effect of aloe has been reported 5 decades back. Since then, more than 300 species of aloe has been identified and researched for various purposes including anti-inflammatory effect<sup>15,16</sup>. Hemorrhoid is characterized by severe inflammation in the venous plexus of the recto-anal region. It is often associated with anal itch, severe pain while defecation and

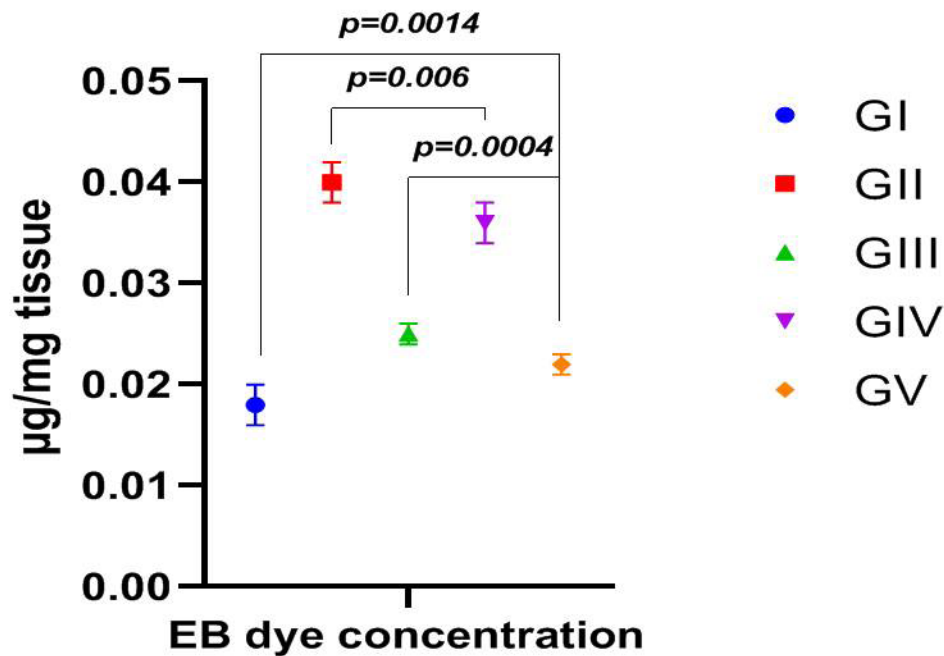
bleeding during defecation which significantly alters the quality of life<sup>17</sup>. Furthermore, hemorrhoid disease does not have a proper treatment so far. Grade III and grade IV hemorrhoid can be surgically managed. Pilex is an ayurvedic formulation which contains *Terminalia chebula*, *Cassia fistula*, *Emblia officinalis* which may improve bowel evacuation and also act as mild laxative<sup>18</sup>. Pilex ointment was used as a standard drug in this study. croton oil has been widely used to



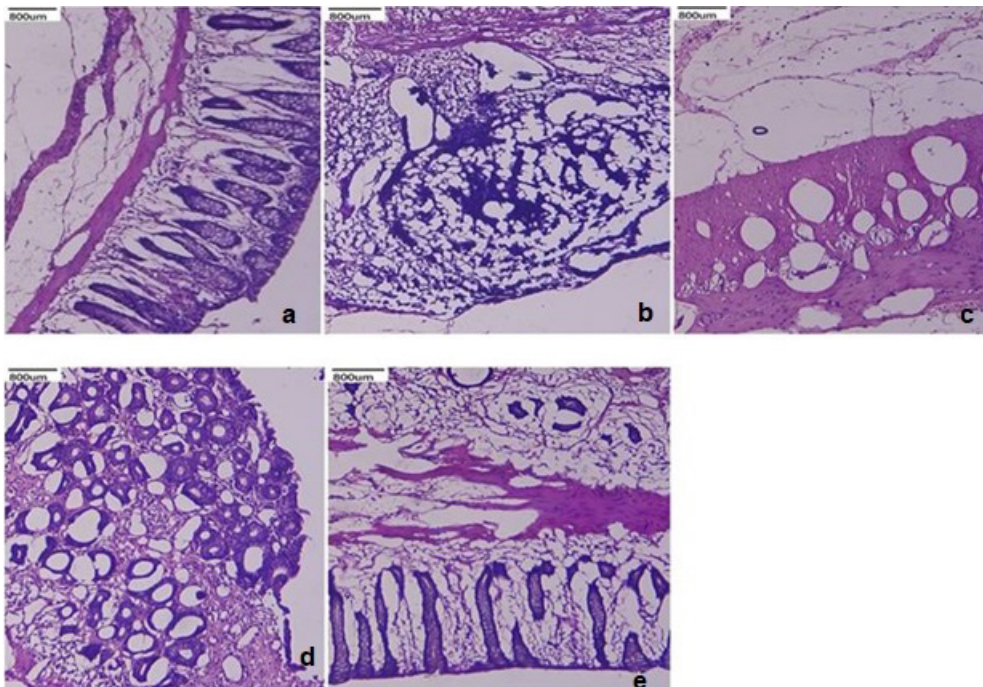
**Figure 3.** Effect of *Aloe Vera* (oral and topical administration) on serum TNF- $\alpha$ . Values are expressed as mean  $\pm$  SEM. Mean values are statistically compared using one way analysis of variance (ANOVA). Any two groups were compared using unpaired *t*-test.  $P < 0.05$  was considered to be statistically significant.



**Figure 4.** Effect of *Aloe Vera* (oral and topical administration) on IL-6. Values are expressed as mean  $\pm$  SEM. Mean values are statistically compared using one way analysis of variance (ANOVA). Any two groups were compared using unpaired *t*-test.  $P < 0.05$  was considered to be statistically significant.



**Figure 5.** Effect of *Aloe Vera* (oral and topical administration) on EB dye concentration extracted from isolated recto-anal tissue. Values are expressed as mean  $\pm$  SEM. Mean values are statistically compared using one way analysis of variance (ANOVA). Any two groups were compared using unpaired *t*-test.  $P < 0.05$  was considered to be statistically significant.



**Figure 6.** a) Photomicrograph of histological section of recto-anal tissue under 40X magnification from GI shows no evidence of ulceration or granulation. b) GII - Ulcerated mucosa, inflammatory granulation tissue with exudates. c) GIII - Mucosal ulceration with mild granulation tissue formation. d) GIV - Mucosal ulceration with dense chronic inflammation and granulation tissue. No evidence of dysplasia or malignancy. e) GV - No evidence of active inflammation or ulceration shows near normal architecture.

induce hemorrhoid in rat<sup>19</sup>. This method is found to be efficient as hemorrhoid is induced in few hours. In the present study, hemorrhoid was induced by croton oil preparation as per previously reported method. Croton oil induces vascular inflammation and cellular infiltration which ultimately results in edema and chemotaxis of neutrophils and macrophages. This results in vascular edema of recto-anal region when applied topically which culminates in hemorrhoid within 6 hours. Croton oil contains phorbol esters such as tetradecanoylphorbolacetate which has the potential to up-regulate COX-2 pathway and expression of inflammatory mediators such as COX-2, TNF- $\alpha$ , IL-6, IL- $\beta$  and other chemokines. These pro-inflammatory mediators released enhance the release of histamine, serotonin, elastase and collagenase which ultimately result in increased vascular permeability associated with inflammation<sup>20</sup>.

Efficacy outcome measures in the present study include the measure of inflammatory markers such as IL-6 and TNF- $\alpha$ , concentration of Evans Blue dye extracted from recto-anal tissue and the weight of isolated tissue as a direct indicator of rectal edema which was measured as recto-anal coefficient (RAC)<sup>13</sup>. EB dye tissue extravasation assay is a method being widely used to quantify the inflammation or edema. Evans Blue dye can be used as a marker of albumin as it has the potential to bind to serum albumin crossing the luminal barrier with high affinity<sup>21</sup>. In the present study pure Aloe Vera gel obtained from the plant was used to study its curative effect in hemorrhoid. The gel obtained by scooping off the sap from thick leaves were used in its purest form, was topically applied to GV. GIV received aloe juice which was made by diluting the gel with distilled water in a blender. Excipients were avoided so as to study the direct effect of aloe gel in its purest form in curing hemorrhoid disease. From the results obtained, it can be depicted that gel of ABM in its purest form when applied topically in the ano-rectal region for 5 days shows significant improvement in symptoms of hemorrhoid disease. This can be further proven by its mitigating effect levels of inflammatory markers in GV when compared to GIII. Statistical analysis of the data proves that aloe gel is much more effective when compared to Pilex ointment which was used as standard. However, the oral preparation was not found to be effective in mitigating the disease as

that of standard. Moreover, GIV showed better results when compared to negative control group (GII). Histopathological evaluation of recto-anal tissues shows ulcerated mucosa with inflammatory granulation exudates in negative control group. The histology of GV revealed near to normal architecture of recto-anal tissue which is more convincing that the histology of group that received Pilex ointment (Figure 6).

Several studies have investigated the biological and toxicological properties of Aloe Vera and have proved to have anti-diabetic activity<sup>22</sup>, cell proliferation property, angiogenesis, immune-modulatory, antimicrobial<sup>23</sup>, anti-cancer properties<sup>24</sup> and various skin diseases<sup>25</sup>. An acute toxicity study done on Aloe Vera gel dosed water showed no signs of toxicity at 500mg/kg<sup>26</sup>. Aloe Vera gel and juice used in the present study was well tolerable and animals did not exhibit any kind of toxicity during the study. ABM is of great ethnic importance as this plant is being used for cosmetic preparation as well as wound healing for decades traditionally. Although different pharmacological toxicological studies have been done on ABM, there is no study done so far on curative effect of this herb on hemorrhoids. Aloe formulation is however proved effective in post-hemorrhoidectomy wound recovery<sup>10</sup>. Phytochemical exploration and effectiveness of different species of Aloe on hemorrhoid disease has to be compared to unravel new insights into the plant species.

## 5. Conclusion

*Aloe barbadensis* Miller was found to be effective in curing hemorrhoids when applied topically. However, curative effect of oral juice of Aloe was not comparable to the standard used (Pilex ointment). Biochemical parameters, weight of recto-anal tissue and histopathological analysis reveals that *Aloe barbadensis* Miller has the potential to mitigate inflammation and is much useful in wound healing.

## 6. Acknowledgement

We express our gratitude to all the supervisors and professors of SRM College of Pharmacy, SRM Institute of Science and Technology, Kattankulathur, Tamil Nadu, India who supported the work.



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