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Evaluation of anti-haemorrhoidal activity of chitosan-based hydrogel incorporated with root extract of *Solanum xanthocarpum* plant

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ABSTRACT

Background: Haemorrhoids, a prevalent anorectal condition involves inflammation, vascular congestion and oxidative stress. It needs safer treatment alternative due to the limitations of existing conventional treatments. *Solanum xanthocarpum* shows natural healing potential and chitosan hydrogels improve delivery, offering a safer, effective and patient-friendly approach for managing haemorrhoids. **Objective:** The present study aimed to evaluate the anti-haemorrhoidal activity of chitosan-based hydrogel containing root extracts of *S. xanthocarpum* using a croton oil induced haemorrhoid model in mice. **Methods:** Aqueous and methanolic root extracts of *S. xanthocarpum* were Soxhlet-prepared and formulated in chitosan hydrogel. Haemorrhoids were induced in Swiss mice using croton oil. Anti haemorrhoidal activity was assessed by determining Evans blue exudation, recto anal coefficient (RAC), tumour necrosis factor alpha (TNF α) levels, malondialdehyde (MDA) generation and histopathology analysis of tissue. **Results:** Croton oil induced marked haemorrhoidal inflammation with elevated RAC, Evans blue leakage, TNF-α and MDA. Treatment with both aqueous and methanolic extracts significantly reduced these parameters compared with the negative control group. Chitosan based gel formulations showed greater therapeutic effects than extracts alone. Histopathology confirmed reduced inflammatory infiltration, improved vascular integrity and restoration of mucosal architecture in treated groups. **Conclusion:** Chitosan hydrogel containing *S. xanthocarpum* root extract possesses significant anti-inflammatory and anti-haemorrhoidal activity. Thus, the formulated hydrogel represents a promising natural therapeutic approach for the management of haemorrhoids.

Keywords: *Solanum xanthocarpum*, Haemorrhoids, Chitosan hydrogel, Croton oil-induced model, Anti-inflammatory activity, Oxidative stress.

INTRODUCTION

Haemorrhoids (piles) are a common anorectal disorder affecting about 4.4% of the global population and nearly 40 million individuals in India. They involve swollen and inflamed vascular tissues in the anal region, most frequently seen in people aged 45-65. Symptoms include rectal pain, itching, bleeding, swelling and discomfort, with bleeding being the most prominent complaint [1-6]. The condition arises due to weakening of supporting tissues and displacement of anal cushions, leading to venous distension, thrombosis and prolapse [4, 6-8]. Contributing factors include constipation, diarrhoea, low-fiber diet, obesity, pregnancy, chronic straining and sedentary lifestyle [3, 9,10]. Despite available treatments, 5-10% of affected individuals avoid conventional therapies due to cost, side effects and recurrence, which may worsen the condition over time [3, 10-12]. Current management strategies include topical drugs, venotonics, dietary supplements and surgical or minimally invasive procedures. However, these approaches often lack long-term effectiveness and may cause adverse effects [11,12]. This has led to growing interest in herbal therapies, which offer anti-inflammatory, analgesic, laxative and styptic effects with fewer side effects and lower cost [3, 9, 13-16].

S. xanthocarpum (yellow-berried nightshade), a medicinal plant from the Solanaceae family, has been traditionally used for treating inflammation, pain and digestive disorders. It contains bioactive compounds such as alkaloids, flavonoids, glycosides and saponins, contributing to its anti-inflammatory, antioxidant and analgesic properties [17].

Its root extract, in particular, is considered beneficial in managing piles, although scientific validation remains limited [4, 13].

Chitosan-based hydrogels, derived from natural polysaccharides, are biocompatible, biodegradable and suitable for localized drug delivery. Their mucoadhesive nature and controlled release properties make them ideal for treating haemorrhoids by enhancing drug retention and therapeutic efficacy [18].

This study aims to evaluate the anti-haemorrhoidal potential of aqueous and methanolic root extracts of *S. xanthocarpum*, incorporated into a chitosan hydrogel, using a croton oil-induced haemorrhoid model in Swiss albino mice.

MATERIAL AND METHODS

Materials

Croton oil and TNF-α ELISA kits were procured from Sigma-Aldrich (St. Louis, USA). Pilex ointment (The Himalaya Drug Company, India) was obtained from a local pharmacy. Chitosan, Evans blue dye and all other reagents and solvents used for extraction and analysis were of analytical grade and purchased from HiMedia Laboratories Pvt. Ltd., Mumbai, India.

Collection and authentication of Plant Material

Roots of *S. xanthocarpum* were collected from wastelands near the Purna River region of Navsari district, Gujarat, India. The plant was authenticated by a qualified taxonomist at Navsari Agricultural University. A voucher specimen (BS/CGBIBT/NAU/1/2020–21) was preserved for future reference.

Preparation of plant extract

Collected roots were thoroughly washed to remove adhering impurities, shade-dried and coarsely powdered. The powdered material was subjected to Soxhlet extraction using methanol and distilled water separately. The extracts were filtered through Whatman No. 1 filter paper and concentrated using a water bath until dryness. The dried extracts were stored at 2-8 °C for further use. Both extracts were later subjected to phytochemical screening.

Phytochemical Analysis of Root Extracts

Qualitative phytochemical screening was performed to identify major bioactive constituents such as Steroid, Saponin, Alkaloid, Phenol, Flavonoid and Coumarin using standard procedures [7,16,19]. For the analysis, 0.1 g root extract were taken and dissolved in 10 mL of distilled water in order to get 10 mg/mL concentration.

Preparation of Chitosan based hydrogel

Chitosan hydrogel was prepared by dissolving 3 g of chitosan (75-85% deacetylated) in 100 mL of 5% (v/v) acetic acid solution under continuous magnetic stirring. To this, 5% of the respective plant extract was added and the mixture was stirred for six h to obtain a uniform hydrogel. A plain chitosan hydrogel without extract was also prepared using the same method [20].

Experimental Animals

Healthy adult, Swiss albino male mice, weighting 25-30 g were used for the experiments. Animals were housed at Central Animal Facility of the Maliba Pharmacy College, Uka Tarsadia University under controlled conditions (22 ± 3 °C temperature, 55 ± 5% humidity and 12 h light–dark cycle) with free access to a standard diet of food pellets and tap water *ad libitum*. Experiments were performed in accordance with the guidelines of Committee for the Purpose of Control and Supervision of Experimental Animals (CPCSEA), Ministry of Environment and Forest, New Delhi after seeking approval by the Institutional Animals Ethical Committee (IAEC) with the proposal approval/sanction number MPC-IAEC-13-2020**.**

Experimental protocol: Induction of haemorrhoids and Evaluation of Gel: Animal Grouping and Treatments

A total of 48 mice were randomly divided into eight groups (n = 6) as shown in Table 1.

Haemorrhoids were induced in all groups except the normal control using croton oil, following established procedures [10,21-24]. Croton oil solution was prepared by mixing deionized water, pyridine, diethyl ether and 6% croton oil in diethyl ether in the ratio of 1:4:5:10. Mice were kept on fasting overnight before application of croton oil solution. Mice of each group were sub-divided into two. One of the subgroups received 0.5 mL of 2% Evans blue via tail vein 1 h before induction of haemorrhoids to assess plasma leakage in the recto-anal region due to edema as an indicator of inflammation [16]. For the induction of haemorrhoids, cotton swabs (4 mm diameter) were soaked in the 100 µL of sterile croton oil and the swabs were inserted in the anus/ano-rectal portion (approximately 20 mmm from the anal opening) and placed for 10 seconds. The mice were observed for 7-8 h for the development of edema. Administration of croton oil is done once a day for 4 consecutive days, looking at the development of haemorrhoids. After 24 h of induction, i.e. On 5th day animals from each group were given relevant treatments for 7 days. The dosage used for the treatment is 1000 µg/mL of extract. The dose of Pile cure cream for the treatment of Group VIII mice was chosen as reported previously [10].

Determination of Haemorrhoidal, Biochemical & Histopathological Parameters

On 12th day, blood samples were collected via retro orbital sinus to estimate the level of inflammatory cytokine, TNF α. using Elisa Microplate Reader [7]. Animals were then euthanized and 10 mm of their recto anal tissues were excised, weighed and used for plasma exudation assessment and histopathological analysis.

Estimation of Evans Blue exudation

Plasma exudation in the retro anal tissues was quantified by determining the amount of Evans blue dye in the anal tissue. Evans blue dye from the appropriately weighed tissue were extracted by keeping the tissues on 2 mL formaldehyde and absorbance was taken at 620 nm using UV spectrophotometer (UV 1800, Shimadzu). Evans blue concentration was quantified using standard curve of Evans blue dye and expressed as micrograms of Evans blue per milligram of ano-rectal tissue [7, 10, 16].

Estimation of recto anal coefficient

RAC was calculated by comparing the weight of recto-anal tissue with the body weight of the animal. This parameter served as an indicator of inflammation severity [10]. The recto-anal-coefficient (RAC) was calculated using the formula:

$$\text{The RAC (Recto-anal coefficient)} = \frac{Weight\ of\ ractoanal\ tissue\ (mg)}{Body\ weight\ (gm)}$$

Oxidative stress parameter

Lipid peroxidation was evaluated by estimating malondialdehyde (MDA) levels using the thiobarbituric acid reactive substances (TBARS) method as originally described by Ohkawa *et al*. [25]. Briefly, 4 mL of reaction mixtures were prepared in a tube containing 0.1 mL serum, 0.20 mL of 8.1% sodium dodecyl sulphate (SDS), 1.5 mL of 20% acetic acid (pH 3.5), 0.20 mL of 0.8% thiobarbituric acid (TBA) and 0.7 mL distilled water. The reaction mixtures were incubated in boiling water bath at 95 °C for 1 h followed by immediate cooling under running tap water. To the tubes, 1 mL of water and 5 mL of mixture of n-butanol and pyridine (15:1 v/v) were

added and vortexed. The tube contents were centrifuged at 3500 rpm for 15-20 min, supernatants were aspirated and the optical density were measured at 532 nm. Calculations were done considering the molar extinction coefficient 1.56×10^5.

Estimation of biochemical parameters

Serum TNF-α was determined using ELISA kits following the manufacturer's instructions.

Histopathological analysis

Recto-anal tissues were excised, fixed in 10% formalin, dehydrated and embedded in paraffin [16]. Sections of 4-6 µm thickness were prepared, stained with hematoxylin and eosin and examined under a light microscope [12]. Histopathological assessments were performed by assessing mucosal and submucosal inflammation, congestion, dilatation of blood vessels, haemorrhage and medium to high degrees of necrosis [7, 10, 26].

Statistical Analysis

All experimental data were expressed as mean ± standard deviation (SD). Statistical analyses were performed using SPSS Statistics. Comparisons among multiple groups were carried out using one-way analysis of variance (ANOVA) followed by Tukey's multiple comparison post hoc test to identify significant differences between groups. A value of $P < 0.05$ was considered statistically significant.

RESULTS

Phytochemical analysis

The medicinal properties of the plants are often linked to the presence of these phytochemical compounds. Phytochemical screening confirmed the presence of key secondary metabolites such as alkaloids, flavonoids, phenolics, saponins, steroids and coumarins in both aqueous and methanolic root extracts of *S. xanthocarpum* as can be seen in Table 2.

These compounds are known for their anti-inflammatory, antioxidant and venotonic properties, which are beneficial in haemorrhoidal conditions [21, 27-31].

Induction of haemorrhoids and Evaluation of Gel

Topical application of croton oil successfully induced haemorrhoid-like lesions in mice, characterized by edema, inflammation and bleeding in the recto-anal region. This is attributed to phorbol esters activating protein kinase C, leading to cytokine release, immune cell infiltration and vascular permeability [32-34]. Elevated inflammatory mediators such as TNF-α further confirmed the inflammatory response [21, 35].

Determination of Haemorrhoidal, Biochemical & Histopathological Parameters

Croton oil significantly increased Evans blue dye leakage, recto-anal coefficient (RAC), TNF-α levels and malondialdehyde (MDA) production in the negative control group compared to normal animals. These parameters were evaluated and represented as mean ± SD are shown in the Table 3. The pictorial representations of comparison of all the parameters between all the groups are shown in Figure 1, Figure 2, Figure 3 and Figure 4 respectively.

Treatment with *S. xanthocarpum* extracts, chitosan and their gel formulations significantly reduced all these parameters. Notably, chitosan-based gel formulations exhibited greater therapeutic effects than extracts alone, although slightly less effective than the standard drug.

Estimation of Evans Blue exudation

Evans blue dye leakage was used to assess vascular permeability and tissue edema associated with haemorrhoidal inflammation. It can be seen from the Table 3 and Figure 1 that, the untreated haemorrhoid group that is negative control group showed high dye extravasation, indicating severe inflammation and vasodilation, consistent with earlier reports [4,10,23,24]. Whereas treatment with chitosan, plant extracts and their gel formulations significantly reduced dye leakage compared to the negative control.

One-way ANOVA revealed a highly significant difference among the experimental groups ($F = 293.86$, $P < 0.0001$). Treatment with chitosan, *S. xanthocarpum* root extracts and their chitosan gel formulations significantly reduced Evans blue dye extravasation compared with the negative control group. Among the treatment groups, the aqueous extract-loaded chitosan gel demonstrated the greatest reduction in vascular leakage, followed by the methanolic extract-loaded gel, indicating improved vascular integrity and reduced capillary permeability. The positive control (Pilex cream) also markedly reduced Evans blue dye extravasation. Overall, the *S. xanthocarpum* extract-loaded chitosan gels exhibited pronounced anti-inflammatory and anti-edematous activity, with therapeutic efficacy comparable to the standard treatment.

Estimation of rectoanal coefficient

The recto-anal coefficient (RAC), an indicator of recto-anal edema and inflammation, differed significantly among the experimental groups (one-way ANOVA, $F = 395.37$, $P < 0.0001$). As shown in Table 3 and Figure 2, the negative control group exhibited the highest RAC values, indicating severe recto-anal inflammation and oedema following croton oil induction. Treatment with both aqueous and methanolic root extracts of *S. xanthocarpum* significantly reduced RAC compared with the negative control group, demonstrating attenuation of inflammatory swelling and improvement in recto-anal tissue integrity. The extract-loaded chitosan hydrogels produced greater reductions in RAC than the corresponding crude extracts, suggesting enhanced therapeutic efficacy through improved local delivery and retention of the bioactive constituents. Tukey's multiple comparison test confirmed that all treatment groups showed significantly lower RAC values than the negative control group ($P < 0.001$). No significant differences were observed between the methanolic and aqueous extract groups or between the standard treatment and the extract-loaded hydrogel groups, indicating comparable efficacy among these treatments in restoring recto-anal function and reducing inflammation.

Estimation of TNF-α

TNF-α, a key pro-inflammatory cytokine, was significantly elevated in the negative control group, indicating severe inflammation. Treatment with chitosan gels containing aqueous and methanolic root extracts of S. xanthocarpum markedly reduced TNF-α levels, confirming their anti-inflammatory potential. The aqueous extract gel showed greater suppression, possibly due to better absorption of hydrophilic bioactive compounds [10,24,34,36]. These findings align with previous studies and correlate with histopathological improvements. TNF-α, released by activated immune cells such as neutrophils, plays a crucial role in inflammation and tissue damage, making it a reliable marker [10,24,34,36]. Its reduction suggests that the formulations effectively modulate inflammatory responses and may offer therapeutic benefits.

As shown in Table 3 and Figure 3, serum TNF-α levels differed significantly among the experimental groups (one-way ANOVA, $F = 174.12$, $P < 0.0001$). The negative control group exhibited the highest TNF-α concentration, indicating a pronounced inflammatory response following croton oil-induced haemorrhoids. Treatment with both aqueous and methanolic root extracts of *S. xanthocarpum* significantly reduced TNF-α levels compared with the negative control group. The

reduction was more pronounced in the chitosan hydrogel formulations than in the corresponding crude extracts, with the methanolic extract-loaded hydrogel showing the greatest decrease among the experimental formulations. Tukey's multiple comparison test demonstrated that all treatment groups, except the vehicle control, exhibited highly significant reductions in TNF-α levels compared with the negative control ($P < 0.001$). These findings indicate that the developed formulations effectively suppressed the inflammatory response and exhibited marked anti-inflammatory activity.

Oxidative stress parameter

Malondialdehyde (MDA), a widely used biomarker of lipid peroxidation and oxidative stress, was evaluated to determine the antioxidant effect of the treatments. As shown in Table 3 and Figure 4, MDA levels differed significantly among the experimental groups (one-way ANOVA, $F = 138.87$, $P < 0.0001$). The negative control group exhibited the highest MDA concentration, indicating pronounced oxidative stress following croton oil-induced haemorrhoids. Treatment with both aqueous and methanolic root extracts of *S. xanthocarpum* reduced MDA levels compared with the negative control group, while the chitosan gel formulations produced a greater reduction than the corresponding crude extracts. Tukey's multiple comparison test demonstrated that the methanolic extract-loaded chitosan gel (REMeC), aqueous extract-loaded chitosan gel (REAqC) and the positive control significantly reduced MDA levels compared with the negative control group ($P < 0.05$). In contrast, the vehicle control, methanolic extract and aqueous extract groups did not show significant differences from the negative control. These findings indicate that incorporation of *S. xanthocarpum* extracts into the chitosan gel enhanced their antioxidant efficacy and effectively attenuated oxidative stress associated with haemorrhoidal inflammation.

Histopathology

Effect of different treatments on the different groups of animals on the histology of the recto-anal tissues of croton oil induced haemorrhoids in mice can be seen in Figure 5. Histopathological examination of rectoanal tissue was performed to observe the cellular and structural changes resulting from the treatments.

Normal control animals showed intact mucosa and normal architecture (Figure 5A), while the negative control group exhibited severe damage, including edema, vascular congestion, dilated vessels and intense inflammatory cell infiltration, confirming successful haemorrhoid induction (Figure 5G). The vehicle control showed only mild improvement (Figure 5B).

Treatment with *S. xanthocarpum* extracts improved tissue morphology. The methanolic extract group showed moderate recovery with reduced inflammation (Figure 5C), while its chitosan gel formulation demonstrated better preservation of mucosal and muscular structures (Figure 5D). The aqueous extract group showed enhanced healing with reduced mucosal damage (Figure 5E). Notably, the aqueous extract chitosan gel exhibited near-normal architecture, minimal edema and significantly reduced inflammatory infiltration, indicating superior tissue repair (Figure 5F). The standard drug also showed reduced inflammation and improved mucosal structure (Figure 5G).

Overall, gel formulations, especially the aqueous-based one, markedly improved histological features by reducing inflammation and promoting tissue regeneration. These findings correlate with biochemical results and support strong anti-inflammatory, antioxidant and tissue-protective effects [37].

Table 1: Experimental groups and treatment regimens

No	Groups	Treatment
1	Group I	Normal Control Group containing animals without inducing Haemorrhoids
2	Group II	Vehicle control: Haemorrhoid induced mice treated with vehicle i.e. Chitosan gel
3	Group III	Methanolic extract treated animals (400 mg/kg of body weight)
4	Group IV	Methanolic extract based chitosan gel treated animals
5	Group V	Aqueous Extract treated animals
6	Group VI	Aqueous Extract based chitosan gel treated animals
7	Group VII	Negative control group: Animals with croton oil induced Haemorrhoids without any treatment
8	Group VIII	Positive control: Animals with croton oil induced Haemorrhoids treated with Piles cure cream as standard drug

Table 2: Phytochemical analysis of *S. xanthocarpum* root extracts

Secondary metabolites	Aqueous Root extract	Methanolic Root extract
Steroid	+	+
Saponin	+	+
Alkaloid	+	+
Phenol	+	+
Flavonoids	+	+
Coumarins	+	+

Table 3: Evaluation of haemorrhoidal parameters in experimental groups

S. No.	Group	Group Description	Evan's Blue Extravasation, [µg/mg of tissue]	RAC	TNF α, [Pg/mL]	Malone dialdehyde (MDA) Generation, [µmol/mL]
1	Group I	Control	0.049 ± 0.004***	0.263 ± 0.057***	78.825 ± 1.734***	-0.082 ± 0.030***
2	Group II	VC	0.154 ± 0.006***	0.931 ± 0.071***	224.937 ± 19.533*	0.228 ± 0.016
3	Group III	REMe	0.116 ± 0.013***	0.704 ± 0.050***	173.288 ± 4.133***	0.220 ± 0.018
4	Group IV	REMeC	0.111 ± 0.006***	0.562 ± 0.087***	106.326 ± 2.983***	0.168 ± 0.006***
5	Group V	REAq	0.134 ± 0.003***	0.711 ± 0.024***	157.775 ± 3.109***	0.226 ± 0.038
6	Group VI	REAqC	0.110 ± 0.005***	0.525 ± 0.053***	124.976 ± 3.194***	0.181 ± 0.011*
7	Group VII	NC	0.195 ± 0.000	1.922 ± 0.082	246.457 ± 23.206	0.262 ± 0.040
8	Group VIII	PC	0.077 ± 0.005***	0.585 ± 0.047***	101.305 ± 5.838***	0.036 ± 0.011***

Note: Values are expressed as mean ± SD (n = 6). Statistical analysis was performed using one-way ANOVA followed by Tukey's multiple comparison test. *P < 0.05, **P < 0.01 and ***P < 0.001 compared with the negative control group (Group VII).

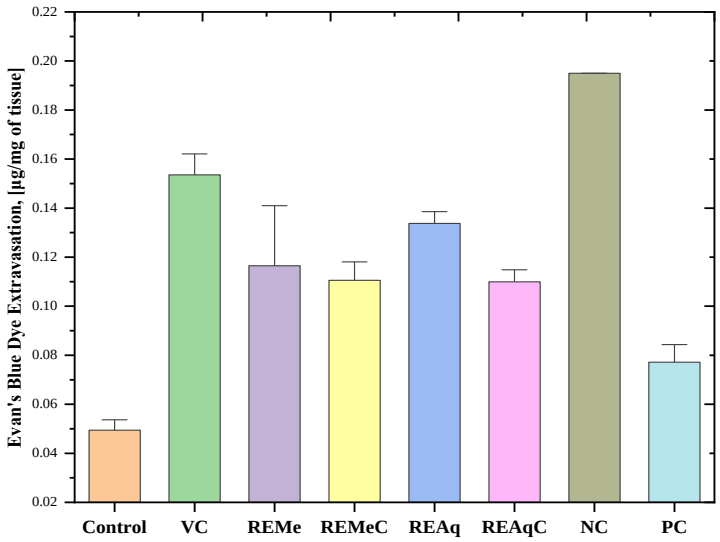

Figure 1: Effect of different treatments on Evans blue dye extravasation in croton oil-induced haemorrhoids in mice. Data are expressed as mean ± SD (n = 6). Statistical analysis was performed using one-way ANOVA followed by Tukey's multiple comparison test. *P < 0.05, **P < 0.01 and ***P < 0.001 compared with the negative control group.

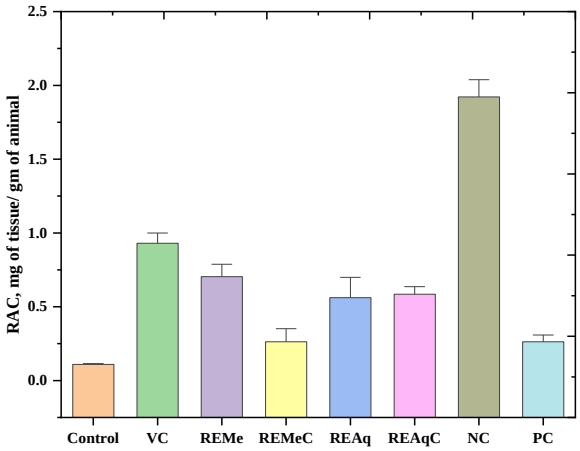

Figure 2: Effect of different treatments on recto-anal coefficient (RAC). Values represent mean ± SD (n = 6). Statistical significance was determined using one-way ANOVA followed by Tukey's post hoc test. *P < 0.05, **P < 0.01 and ***P < 0.001 versus the negative control group.

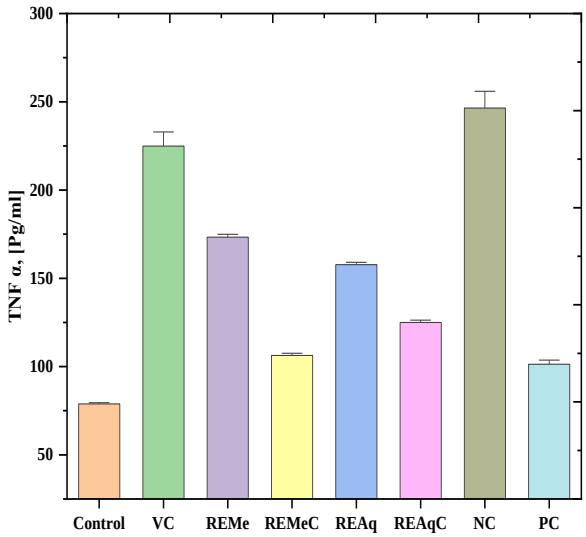

Figure 3: Effect of different treatments on Inflammatory cytokine serum TNF-α levels in croton oil-induced haemorrhoids. Values are presented as mean ± SD (n = 6). Statistical comparisons were performed using one-way ANOVA followed by Tukey's multiple comparison test. *P < 0.05, **P < 0.01 and ***P < 0.001 compared with the negative control group.

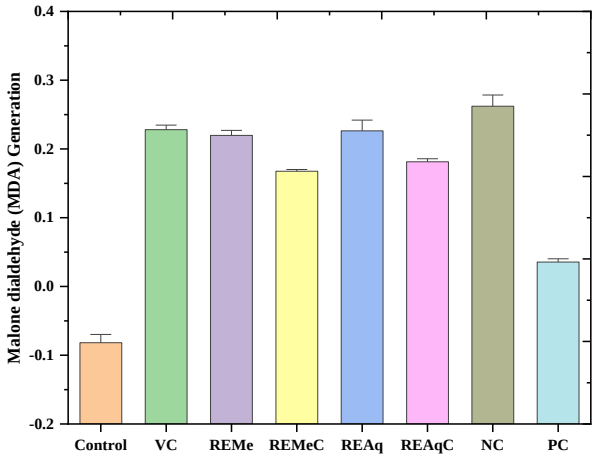

Figure 4: Effect of different treatments on malondialdehyde (MDA) levels. Data are expressed as mean ± SD (n = 6). Statistical significance was determined using one-way ANOVA followed by Tukey's multiple comparison test. *P < 0.05, **P < 0.01 and ***P < 0.001 compared with the negative control group.

DISCUSSION

The present study demonstrated that *S. xanthocarpum* root extracts possess significant anti-haemorrhoidal activity, particularly when formulated as a chitosan-based hydrogel. The croton oil-induced haemorrhoid model successfully reproduced the pathological features of haemorrhoidal disease, including oedema, vascular congestion, inflammatory cell infiltration and oxidative stress. Similar changes have been consistently reported in previous studies employing this experimental model, confirming its reliability for evaluating novel anti-haemorrhoidal therapies [10,21-24,32].

One of the major findings of the present investigation was the significant reduction in Evans blue dye extravasation following treatment with both aqueous and methanolic extract-loaded hydrogels. Evans blue leakage is widely accepted as an indicator of vascular permeability and inflammatory oedema. The observed reduction indicates that the formulations effectively preserved vascular integrity and reduced capillary leakage, thereby limiting tissue swelling. Comparable improvements in vascular permeability have been reported for herbal anti-haemorrhoidal formulations prepared from *Acacia ferruginea, Amorphophallus paeoniifolius* and other medicinal plants, suggesting that stabilization of the microvasculature is an important mechanism underlying their therapeutic activity [7,10,16,24].

Recto-anal coefficient (RAC), which reflects the degree of recto-anal oedema and inflammation, was markedly elevated in the untreated haemorrhoidal group but significantly reduced after treatment. Restoration of RAC indicates improvement in recto-anal tissue integrity and reduction of inflammatory swelling. Similar observations have been reported in experimental haemorrhoid models treated with herbal preparations, where reduction in RAC was associated with improved recto-anal function and tissue recovery [10,16,24]. The aqueous extract-loaded hydrogel showed relatively greater improvement than the methanolic formulation, suggesting that hydrophilic phytoconstituents may contribute more effectively to tissue repair and local therapeutic activity.

Inflammation plays a central role in the pathogenesis of haemorrhoids and TNF-α is one of the major cytokines responsible for amplifying the inflammatory response. In the present study, treatment with *S. xanthocarpum* formulations significantly reduced serum TNF-α levels compared with the untreated group, indicating effective suppression of inflammatory signalling. Histopathological examination further supported these findings by demonstrating reduced inflammatory cell infiltration, decreased oedema, minimal vascular congestion and restoration of normal mucosal architecture in treated animals. Similar reductions in TNF-α and improvements in tissue morphology have previously been reported for several herbal anti-haemorrhoidal formulations, confirming that modulation of inflammatory mediators is a major mechanism responsible for their therapeutic effects [10,12,22,24,34,36].

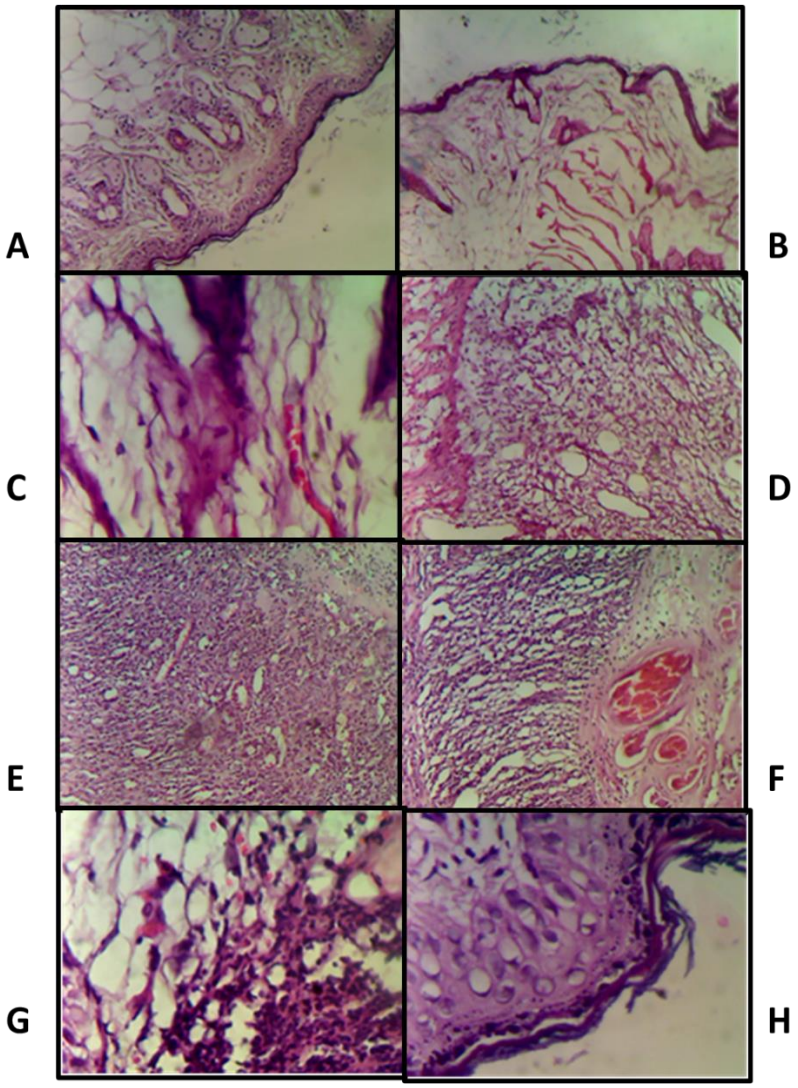

Figure 5: Effect of chitosan gel formulations on the histology of the recto-anal tissues of croton oil induced haemorrhoids in mice

a: Normal control group showed intact and normal recto-anal histology
b: Vehicle control group showed slight reduction in inflammation compared to the negative control
c-f: Treatment groups (Methanolic extract, Chitosan-based methanolic gel, Aqueous extract, and Chitosan-based aqueous extract) showed mild improvement in piles condition
g: Negative control group showed severe edema in tunica serosa, hyperemia, and marked submucosal vessel dilatation
h: Standard drug (Piles cure cream) showed mild to moderate infection with mild congestion

The anti-inflammatory activity observed in the present study is likely related to the phytochemical composition of *S. xanthocarpum* root extracts. Qualitative phytochemical analysis confirmed the presence of flavonoids, phenolic compounds, alkaloids, saponins, steroids and coumarins. These bioactive constituents have been widely reported to inhibit the production of pro-inflammatory cytokines, suppress inflammatory enzyme activity and improve vascular stability. Flavonoids and phenolic compounds, in particular, possess strong free radical scavenging activity and have been shown to improve venous tone and capillary resistance, which are beneficial in the management of haemorrhoids [17,27-31]. The combined action of these phytochemicals is therefore likely responsible for the broad anti-inflammatory and tissue-protective effects observed in the present investigation.

Oxidative stress is another important factor contributing to haemorrhoidal tissue injury. Excessive generation of reactive oxygen species promotes lipid peroxidation, leading to membrane damage and delayed tissue repair. In the present study, malondialdehyde (MDA), a well-established marker of lipid peroxidation, was significantly reduced in animals treated with the extract-loaded hydrogels, indicating effective attenuation of oxidative stress. These findings are consistent with previous reports demonstrating the antioxidant activity of *S. xanthocarpum* and other medicinal plants rich in flavonoids and phenolic compounds [16,28,31]. Gulati *et al*. [37] also reported that aqueous extracts of *S. xanthocarpum* significantly reduced inflammatory responses and oxidative stress in experimental models, supporting the observations of the present study.

An important outcome of this investigation was that both extract-loaded hydrogels produced greater therapeutic effects than the corresponding crude extracts. This improvement can be attributed to the physicochemical properties of chitosan, which is a biodegradable, biocompatible and mucoadhesive polymer capable of prolonging residence time at the site of application and providing sustained release of incorporated phytoconstituents. Enhanced local retention and controlled drug release are likely to improve the bioavailability of active compounds within the inflamed recto-anal tissues, thereby producing superior therapeutic effects compared with administration of the extracts alone [18,20]. These characteristics make chitosan an attractive carrier for localized treatment of haemorrhoidal disease.

The superior performance of the aqueous extract-loaded hydrogel throughout the study deserves particular attention. It consistently demonstrated better reduction in vascular permeability, inflammatory biomarkers and oxidative stress while producing greater restoration of tissue architecture. This enhanced efficacy may be attributed to a higher concentration of hydrophilic bioactive constituents that are

more readily released from the chitosan matrix and more effectively distributed within the affected tissues. Consequently, the aqueous formulation may represent a more suitable candidate for further pharmaceutical development.

The findings of the present study provide scientific evidence supporting the traditional use of *S. xanthocarpum* in the management of haemorrhoids. More importantly, they demonstrate that combining herbal extracts with a chitosan-based drug delivery system substantially improves therapeutic performance. Such localized formulations may offer several advantages over conventional therapies, including prolonged drug retention at the site of action, reduced dosing frequency, improved patient compliance and fewer systemic adverse effects. Besides haemorrhoids, these formulations may also have potential applications in other inflammatory conditions involving impaired tissue healing, although dedicated studies are required before such applications can be confirmed.

Despite these encouraging findings, certain limitations should be acknowledged. The study was conducted in an acute experimental animal model using a relatively limited sample size. The active phytoconstituents responsible for the observed pharmacological effects were not quantitatively characterized and the molecular mechanisms were investigated primarily through TNF-α without evaluating other inflammatory pathways such as NF-κB, IL-1β or IL-6. Therefore, future studies should focus on phytochemical standardization, molecular mechanism studies, formulation optimization, long-term toxicity assessment and well-designed clinical trials to establish the safety and therapeutic efficacy of these formulations in human subjects.

Overall, the present findings indicate that chitosan-based hydrogels incorporating *S. xanthocarpum* root extracts possess significant anti-inflammatory, antioxidant and tissue-protective properties. By reducing vascular permeability, suppressing inflammatory mediators, limiting oxidative stress and promoting tissue repair, these formulations demonstrate considerable potential as a natural therapeutic approach for the management of haemorrhoidal disease. The aqueous extract-loaded hydrogel consistently exhibited superior efficacy, making it a promising candidate for further preclinical and clinical development.

CONCLUSION

This study provides scientific validation for the traditional use of *S. xanthocarpum* in haemorrhoid management. Chitosan-based gel formulations containing root extracts demonstrated significant anti-inflammatory, antioxidant and tissue-protective effects in a croton oil-induced haemorrhoid model. The formulations effectively reduced vascular permeability, inflammatory markers and oxidative stress while improving tissue architecture. Among them, the aqueous extract gel showed comparatively superior efficacy. The results suggest that chitosan-based herbal gels can serve as a promising, natural and effective therapeutic approach for haemorrhoids. Further studies on formulation optimization and clinical evaluation are recommended to confirm their applicability in humans.

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Author Contributions

US and DP conceptualized the study and the manuscript. SB was involved in the methodology, literature review, and execution of the research work. SB and SP carried out the investigation. BV performed the formal statistical analysis and contributed to data acquisition. SP was responsible for the histopathological examination and interpretation of histological findings, while BV conducted the hematological examination. US, DP, and BV were involved in data curation. SB and SP prepared the original draft of the manuscript. DP and US reviewed and edited the manuscript. DP, US, and BV supervised the study. All authors have read and approved the final version of the manuscript.

Data Availability Statement

The data that support the findings of this study are available and will be made available upon request.

Use of AI in Drafting of Manuscript

The authors declare that they have not used any generative AI/AI-assisted technologies in the writing of this manuscript.

Conflict of interest

The authors declared no conflict of interest.

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