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Research Article

Phytochemical Standardization of *Semicarpus Anacardium* and Evaluation of Anti-haemorrhoidal Activity in Animal Model

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A B S T R A C T

Many herbal remedies individually or in combination have been recommended in various medical treatments for the cure of different diseases. *Semecarpus anacardium* (SA) Linn (Family: Anacardium), is a plant well known for its medicinal value in ayurvedic and siddha system of medicine. The *Semecarpus anacardium* nuts are used for various medicinal and pharmacological purposes from ancient period. It is well proved that hemorrhoids are pathological conditions. In these conditions there occurs vasodilation at recto anal portion at severe level. This promotes the inflammation of surrounding tissues, further leading to secondary difficulties. These are extravasation of fluid into interstitial space that is due to increased vascular permeability and migration of large quantity of inflammatory cells (granulocytes and monocytes). In the present investigation, croton oil has been used as inducer/phlogistic agent to induce experimental hemorrhoids. Croton oil causes severe inflammation due to the release of inflammatory lipid metabolites likes (prostaglandins, leukotrienes, lipoxins) cytokines (TNF- α and IL-6). These factors, alone or in combination, regulate the activation of resident cells (fibroblasts, endothelial cells, macrophages and mast cells).

Keywords: *Semicarpus Anacardium*, Phytochemical standardization, Anti Haemorrhoidal Activity, Animal Model, Herbal formulation, Ointment

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INTRODUCTION:

Hemorrhoids are anorectal disorders that affect about 4.4% of the world's population and are more common in people between the age group of 45–65 years. Studies have revealed that, around 5–10% affected patients from hemorrhoids hesitate to receive conventional treatments [1]. The risk factors associated with the changes in pathophysiological condition of anal hemorrhoid includes constipation, diarrhea, alcohol consumption, aging, low-fiber diet, spicy diet and chronic straining. Major symptoms associated with hemorrhoid complaints include inflammation, pain, bleeding and pruritus [2]. Treatment available for hemorrhoids mainly includes cryotherapy, laser treatment, sclerotherapy, hemorrhoidectomy, infrared photocoagulation and bipolar diathermy. Generally, these therapies are very expensive and have several side effects with no total recovery. Therefore, researchers are in search of a treatment that may provide complete relief with less cost and low side effects. Hemorrhoids are not varicosities; they are clusters of vascular tissue (eg, arterioles, venules, arteriolar-venular connections), smooth muscle (eg, Treitz muscle), and connective tissue lined by the normal epithelium of the anal canal. Hemorrhoids are present in utero and persist through normal adult life. Evidence indicates that hemorrhoidal bleeding is arterial and not venous. This evidence is supported by the bright red color and arterial pH of the blood [3]. Treatment of hemorrhoids in modern medicine is still in infancy. Given the fact that there is no specific drug to treat hemorrhoids, extensive research is ongoing in the field of Ayurveda for utilizing the natural sources for treating hemorrhoids. Pilex, a polyherbal proprietary formulation of The Himalaya Drug Company (Bangalore, India), is used clinically over the years for the treatment of hemorrhoids and other related conditions. Several reports are available indicating the beneficial effects of Pilex [4-5]. natural products have been used to treat many clinical ailments as described in different traditional systems of medicine worldwide since antiquity [6]. Among these, Ayurveda is one of the traditional medicine systems prevalent in the Indian subcontinent, which describes the holistic approach to treating various human clinical ailments, including processed medicinal plants, some animal parts, and minerals [7]. Therefore, medicinal plants described in Ayurveda might serve as a potential source for rational drug design for many diseases, including cancer, neurological disorders, and lung and liver diseases. Many phytoconstituents from medicinal plants contain potential bioactive molecules *Semecarpus anacardium* is rich in various bioactive substances, such as flavonoids, biflavonoids, phenolic compounds, andaloids. Notable components like anacardic acid, bhilawanols, and semicarpol have been recognized as contributing to its biological functions. These phytochemicals are known for their strong antioxidant, anti-inflammatory, and antimicrobial characteristics, making the plant valuable for a range of therapeutic uses [8]. Phytochemical analysis of *Semecarpus anacardium* nut shows biologically active compounds such as biflavonoids, phenolic compounds, bhilawanols, minerals, vitamins and amino acids, which shows various medicinal properties. Traditional healers and physicians use formulations of *Semecarpus anacardium* in their clinical practice. On the basis of previous reported research work and traditional uses, the present study was aimed to

formulation of ointment containing *Semicarpus anacardium* and evaluate for anti-hemorrhoids activity.

MATERIAL AND METHODS:

Extraction & Phytochemical Screening: All chemicals and solvents were of analytical grade (AR Grade) and were purchased from Sigma Aldrich, Ranbaxy fine chemicals Ltd., LOBA chemicals Ltd., s.d. fine chemicals Ltd., Spectrochem chemicals. Pre-coated TLC plates having silica gel 60 F254 thickness 0.2 mm were purchased from Merck. All the solvents used for HPLC analysis were purchased from JT Baker and Fischer scientific Ltd.

Collection and authentication of plant material: The selected seed material of *Semicarpus anacardium* were purchased from local market of Kanpur India, and authenticated.

Macroscopic studies: The selected crude drugs were subjected to studies organoleptic characters viz., color, odour, appearance, taste, texture etc.

Physicochemical Evaluation: Physiochemical qualities, for example, ash values and extractive values were researched for chose plant as the official strategies and as per WHO guidelines. Ash values (Total ash, Acid insoluble ash and Water-soluble ash), Extractive values, Loss on drying and pH were determined.

Extraction Process of Drug: Extraction includes partition of bioactive segment of the plant tissues from the latent moiety by utilizing specific solvents in standard extraction systems. Plant herbs were extracted successively with hexane, and methanol utilizing maceration method of extraction. The totally dried leaves of *Semicarpus anacardium seed* was coarsely powdered and afterward extracted with non-polar solvent hexane for defatting of plant material. Seed powder (200g) was stuffed in vessel and kept with hexane for 24 hours and procedure was repeated till complete extraction. The plant material then kept with methanol for 24 hours and procedure was repeated till complete extraction. The obtained methanol extract was filtered and concentrated on rotatory evaporator to get methanol extract.

Preliminary phytochemical analysis of extracts: Qualitative test as phytochemical examination of any plant species is a vital procedure as it gives the starter data about presence of different chemical constituents and furthermore gives further possibilities of the specific plant species in its future research examinations. The extracts acquired by extraction methods were exposed to different chemical tests to recognize the presence of a class of chemical constituents.

Preparation of Ointments: Three topical ointment bases of varying degrees of aqueous/anhydrous character namely simple emulsifying wax, white soft paraffin and liquid paraffin were prepared by fusion method. In this method the constituents of the base were placed together in a melting pan and allowed to melt together at 70°C. After melting, the ingredients were stirred gently maintaining temperature of 70°C for about 5 minutes and then cooled with continuous stirring. Formulation of ointment done by incorporating 10 g of the semisolid methanolic extract of *Semicarpus anacardium seed* into the various bases by triturating in a ceramic mortar with a pestle to obtain 100 g of herbal ointments containing 10 % w/w of *Semicarpus anacardium seed***.** The prepared herbal ointments were put in ointment jars, labeled and were stored at room temperature.

Table 1: Composition of the methanolic extract of *Semicarpus anacardium* seed ointment

S. No.	Components	Amount (gm)
1	Methanolic extract of *Semicarpus anacardium* seed	1
2	Emulsifying wax	5
3	White soft paraffin	2
4	Liquid paraffin	2

Evaluation of ointment: The evaluations were carried out on the ointment by using the following parameters.

Colour and odour: Colour and odour of ointment, examine by visual examination.

Loss on drying: 1 g of ointment was placed in the Petridis and heated in the water bath at 105 °C every 30 min until it gets constant weight.

pH: The pH of ointment was determined by digital pH meter. 1 g of ointment was dissolved in 50 ml of distilled water and the pH was measured.

Diffusion study: The diffusion study was carried out by preparing agar nutrient medium of any concentration. It was poured into petridis. A hole bored at the center and ointment was placed in it. The time taken for the ointment to get diffused was noted.

Animal Studies (Anti haemorrhoidal activity):

Croton-oil induced hemorrhoid model: The overnight fasted rats were initially injected with Evans blue (EB) dye (Sigma Aldrich.) via tail veins, which act as a marker for judgement of inflammatory index. Half an hour after the injection of EB dye, hemorrhoids were induced in rats by application of croton oil preparation, prepared by mixing deionized water, pyridin, diethyl ether (S D Fine-Chem Limited, Mumbai, India) and 6% croton oil (Sigma Aldrich, St, Louis Mo. U.S.A.) in diethyl ether in the ratio of 1: 4: 5: 10. Except normal group, the croton oil preparation was inserted in the rectoanal portion of the rats, 20 mm deep into the anal opening of rats, using sterile cotton swabs having diameter of 4 mm, soaked in 100 µl of prepared croton oil. After 7 to 8 hrs of croton oil application, the animals were observed for any development of edema Anti-hemorrhoidal activity of ointment formulation containing extract of *Semicarpus anacardium* seed. Wistar rats were divided into five groups as given below:

Group I was indicated as normal control and was applied topically simple ointment base twice daily for 5 days.

Group II marked as positive control and administered with 6% croton oil, 100µl/day into the recto-anal region for 3 days).

Groups III, was given topically TPMA (ointment containing *Semicarpus anacardium* seed extract twice daily after 3 days of 6% croton oil (100µl/day) treatment.

Groups IV, was indicated as standard group and given topically Himalaya Pilex Ointment (30 gm) twice daily after 3 days of 6% croton oil (100µl/day) treatment.

By administering croton-oil preparation to all groups except the usual control group, haemorrhoids were produced (croton oil preparation: deionized water, pyridine, diethyl ether, and 6 percent croton oil in diethyl ether with ratio of 1: 4: 5: 10). A sterile cotton swab (4 mm diameter) was placed into the anus (about 22 mm diameter) and held for 10 seconds after being soaked in cotton oil preparation (100µl). A linear development of oedema was observed within 7-8 hrs of induction of croton oil. After 24h of induction, relevant treatment was given to all the groups for five days. On fifth day, 1h after the treatment, blood samples were collected from the retro-orbital sinus and inflammatory cytokines like PG, TNF-α and IL-6 were estimated in blood by using Elisa Microplate Reader (Erba Lisa Scan EM, Mumbai). All animals were euthanized by exsanguinations under deep isoflurane anesthesia and rectoanal tissue (20 mm in length) was isolated and weighed. For histological examination, same tissue was examined for severity score and rectoanal coefficient by fixing the tissue in 10% neutral buffered formalin. The rectoanal-coefficient (RAC) was calculated from the formula:

Rectoanal Coefficient = Weight of rectoanal tissue (mg) / Body weight (mg)

Histological observation was performed for inflammation, congestion, hemorrhage, vasodilation and necrosis.

RESULTS AND DISCUSSION

Collection and authentication of plant material: The selected plant material *Semicarpus anacardium seed* were purchased from local market Kanpur India, and authenticated.

Macroscopic studies: A systematic approach is necessary in pharmacognostic study, which helps in confirmation and determination of identity, purity and quality of a crude drug. The selected crude drugs were subjected to studies organoleptic characters viz., color, odour, appearance,

taste, texture etc. The nut is about 25 millimetres (1 in) long, ovoid and smooth lustrous black, while the reddish-orange accessory fruit is edible and sweet when ripe. However, the black fruit and its resin are toxic and can cause severe allergic reactions

Physicochemical Evaluation: Physicochemical parameter such as Ash values (Total ash 12.01, Acid insoluble ash 0.21 and Water-soluble ash 6.1), All parameters of selected drugs found within the limit as per API. The extractive values are mainly useful for the determination of adulterated or exhausted drug. Alcohol soluble extractive value of *Semicarpus anacardium* seed were found 14.12 % w/w whereas Water soluble extractive value was found 18.33 % w/w.

Extraction process of drug: Methanol extract of *Semicarpus anacardium* seed drugs obtained by maceration method.

Preliminary phytochemical analysis of extracts: Extract (methanol extract) of selected plant drugs obtained by maceration method was subjected to qualitative phytochemical tests to identify the presence of secondary metabolite (viz., alkaloids, glycosides, tannins, flavonoids, sterols, fats, oils, phenols and saponins) present in them. Preliminary phytochemical screening of methanol extract exhibited the presence of carbohydrate, tannin flavanoid, glycoside and saponin in methanol extract (Table 2)

Table 2: Phytochemical analysis of *Semicarpus anacardium* seed extracts

S. No.	Phytochemical	Indication test	Methanol extract
1	Alkaloid	Dragendorff test	+
2	Napthoquinon	Juglone test	_
2	Steroid	Salkowaski test	-
3	Carbohydrates	Molish test	+
4	Triterpene	Vanillin-sulphuric acid test	-
5	Tannin	Ferric chloride test	+
6	Glycosides	Keller-killani test	+
7	Protein	Biuret test	-
8	Flavonoid	Shinoda Test	+
9	Saponin	Lead acetate test	+

Where + is Present and – is Absent

Evaluation of herbal ointment: Ointments are homogeneous, semi-solid preparations intended for external application to the skin or certain mucous membranes for emollient, protective, therapeutic or prophylactic purposes where a degree of occlusion is desired. They usually consist of solutions or dispersions of one or more medicaments in suitable bases. They are formulated using hydrophobic, hydrophilic or water-emulsifying bases to provide preparations that are immiscible, miscible or emulsifiable with the skin secretion, respectively. The base should not produce irritation or sensitization of the skin, nor should it retard wound healing. It should be smooth, inert, odorless or almost odorless, physically and chemically stable and compatible with the skin and with incorporated medicaments. The proportion of the base ingredients should be such that the ointment is not too soft or too hard for convenient use. The consistency should be such that the ointment spreads and softens when stress is applied.

Table 3: Physicochemical parameters of *Semicarpus anacardium* seed herbal ointment formulation

S. No	Physicochemical parameters	Observation
1	Colour	Dark greenish
2	Odour and taste	Characteristic
3	Loss of drying	0.16%
4	pH	6.4
5	Diffusion study	2 cm in 1 min

Anti haemorrhoidal activity: Hemorrhoids are well-known to be pathological disorders consists vasodilation in the recto anal region occurs at a severe level in these circumstances. This causes inflammation in the surrounding tissues, which leads to additional complications. Extravasation of fluid into the interstitial space as a result of enhanced vascular permeability and migration of a significant number of inflammatory cells are two examples (granulocytes and monocytes). In the present investigation, croton oil has been used as inducer/phlogistic agent to induce experimental hemorrhoids. Croton oil was employed as an inducer/phlogistic drug to generate experimental haemorrhoids in the current study. Croton oil triggers severe inflammation by releasing inflammatory components such as leukotrienes, prostaglandins and lipoxins, as well as cytokines such as TNF- and IL-6. These variables, individually or in combination, control the activation of resident cells (fibroblasts, endothelial cells, macrophages, and mast cells) as well as newly recruited inflammatory cells (monocytes, lymphocytes, neutrophils, and eosinophils), resulting in a systemic inflammatory response).

Effect of prepared herbal ointment formulations on Severity score and Recto Anal Co-efficient (RAC) in Croton oil induced hemorrhoid: One way for assessing the risk for harm in hemorrhoidal rats during defecation, comparing therapies, monitoring disease, and assisting surgical decisions is to use a severity score. This is a method for describing wistar rats with multiple injuries and evaluating emergency care. Severity score is number of injuries in recto anal portion of wistar rats.

All rats were scored with the Sodergren score prior to receiving a standardized treatment regime. Sodergren score is the base line of injuries before treatments. The nature and severity of the rat's hemorrhoidal symptoms have been considered as the pre-treatment baseline. A follow up period: 5 days was carried out to study post treatment screening, to find out whether the results are pass or fail. The data collected and compared with positive and negative control group statistically.

Results revealed no severity in normal control group and the severity score of positive control group was found to be 0.176±0.05 and 1.826±0.17, respectively, which is statistically significant in comparison to normal group. Interestingly, treatment with SAHO Ointment (5%w/w) has remarkably decreased as 0.216±0.05, for SAHO ointment, respectively.

So base line for severity score has been considered as zero (0) for control group and has been find out for hemorrhoid induced wistar rats 1.826 in positive group. It has been find out that severity score in hemorrhoid induced wistar rats treated with SAHO Ointment were reduced and SAHO Ointment showed approximately near to the standard group treatment. This indicates that herbal ointment of *Semicarpus anacardium* seed is able to treat injuries produced due to hemorrhoids similar to Himalaya Pilex Ointment.

Table 4: Effect of prepared herbal ointment formulations on histomorphological score of hemorrhoidal ano-rectal tissues

Animal groups	Severity score	Rectoanal Coefficient
Normal Control (vehicle)	0.176±0.05	0.426±0.07
Control (Croton oil 100µl/day)	1.826±0.17	1.683±0.03
Croton oil + Herbal Ointment (SAHO) (5%w/w)	0.189±0.02	0.462±0.08
Croton oil + Himalaya Pilex Ointment (30 gm)	0.220±0.07	0.492±0.10

Each value is the mean ± S.D. (n = 5), SAHO: Ointment containing *Semicarpus anacardium* seed extract

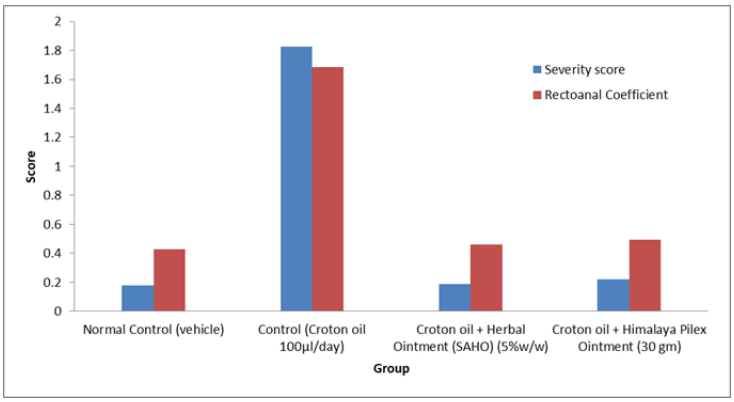

Figure 1: Effect of prepared herbal ointment formulations on Severity score and Rectoanal Coefficient of hemorrhoidal ano-rectal tissues

Effect of different extracts on pro-inflammatory mediators in Croton oil induced hemorrhoid: Croton oil application in the rectoanal region induces hemorrhoid characterized by the marked expression of some pro-inflammatory cytokines. Inhibitory effect of flavonoid rich extracts on the tissue level of pro-inflammatory mediators (TNF-α, IL-6 and PGE2) were measured in rectoanal tissues of rats by ELISA. Results were confirmed that Herbal Ointment (SAHO) treatment groups were showed significant decrease in the tissue level of proinflammatory mediators i.e. TNF-α, IL-6 and PGE2 when compared to the positive control group of animals. Herbal Ointment (SAHO) (5%w/w) have significant effect on tissue cytokines level such as TNF-α, IL-6 and prostatglandin E2. Effect of Herbal Ointment (SAHO) (5%w/w) was comparable to the standard treated group and approximate near to the normal control group. Herbal Ointment (SAHO) (5%w/w) treated group also maintained Severity score and rectoanal coefficient near to the normal control group of animals

SUMMARY AND CONCLUSION:

Hemorrhoid is one of the most common inflammatory diseases which can be characterized by alteration in vasculature of the anal canal including blood vessels, supporting tissues, muscles and elastic fibers. Croton oil application in the rectoanal region induces hemorrhoid characterized by the marked expression of some pro-inflammatory cytokines. Inhibitory effect of flavonoid rich extracts on the tissue level of pro-inflammatory mediators (TNF-α, IL-6 and PGE2) were measured in rectoanal tissues of rats. Results were confirmed that herbal ointment treatment groups were showed significant decrease in the tissue level of proinflammatory mediators. It has been find out that severity score in hemorrhoid induced wistar rats treated with SAHO Ointment were reduced and SAHO Ointment showed approximately near to the standard group treatment. This indicates that herbal ointment of *Semicarpus anacardium* seed is able to treat injuries produced due to hemorrhoids similar to Himalaya Pilex Ointment.

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