MANAGEMENT OF INTERNAL HEMORRHOIDS BY KSHARA KARMA; A HISTOLOGICAL STUDY

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ABSTRACT

“Kshara karma (application of caustic alkaline paste) has been used in Ayurveda to treat Hemorrhoids from Sushruta period. Arsha (hemorrhoids) is commonly resides in anal region (Gudasthan) mostly at 3, 7 & 11’o’ clock position with bleeding tendency. In modern era Kshara karma is not very popular procedure among the physicians and study about this is very less especially at histological level. Therefore, the present work carried out an experimental work in vivo in a mouse model to see the effect of “Kshara karma” by evaluating the histological sections performed at the, 2nd, 4th, 6th and 8th days after treatment with Kshara (application of caustic alkaline paste) and it’s neutralized with lime fruit juice. Because Teekhna kshara plays pivotal role for making famous Ayurvedic Surgeon in the aspect of treating piles mass. The histological sections were analyzed with regard to inflammatory response and vascularization followed by liberation of slough tissue and healing with granulation and fibrosis of the local area of affection at the end. Kshara karma displayed increased Inflammation with vascularization during the first 6 days of treatment compared to the control animals treated with normal saline the same time period. The number of inflammatory cells (neutrophils) was dissimilar in both groups during the period, significantly reducing at the end of inflammatory phase (3rd day of treatment). The reduction is consistent with the progression of healing process from 6th to 8th day. In addition, the density of blood vessels was also statistically dissimilar in both matrices.

KEYWORDS: Arshas, Internal hemorrhoids Kshara karma, Piles, Swiss albino mice.
INTRODUCTION

Arshas (piles) which are a varicosity of hemorrhiodal veins in anal region common problem in society now a days, due to more intake of spicy and chilly foods and irregular food intake habits, sedentary life style, long drive of vehicles, mental stress which Increases the rate of incidence of this disease. Hemorrhoids occur frequently in the adult population. Notably, a considerable number of people with hemorrhoids do not complain about symptoms. Hemorrhoids that causes problem are found in the ratio of 2:1 of men and women. Different treatment modalities to cure hemorrhoids like expectant medical therapy, injection treatment, rubber band ligation, manual dilatation, cryosurgery, infrared coagulation, and operative treatments like formal hemorrhoidectomy are available. However, the risk of recurrence or of developing an infection of the wound after the operation is high. Risk or complications of Milligan-Morgan hemorrhoidectomy are pain, retention of urine, secondary hemorrhages and development of abscess and fistula. Pain which varies virtually from patient to patient, either nil to severe pain, retention of urine in which 7% of patients required catheterization, secondary hemorrhages - 1.2% of patients, development of abscess or fistula (53.57%). Kshara karma enumerated best among four treatment modalities of hemorrhoids as mentioned in classical Ayurvedic texts, but it is not widely practiced in all over India. Kshara karma (application of Pratisaraneeya teekshna kshar): It is a non-surgical procedure of Ayurveda indicated for the management of hemorrhoids. A medicine (alkaline in nature) derived from a combination of various herbs is applied to the pile mass with the help of a special slit proctoscope. The preparation of teekhna kshar is described by various types plants and by burning total leaf, fruit, stems and roots of Kutaja, Palasha, Aswakarna, Paribhadra, Bibhitaka, Aragvadha, Snuhi, Apamarga, Gunja etc. It causes sloughing action of local tissue with a black berry discoloration and inflammation followed by healing with fibrosis. It is a type of chemical cauterizer. The Kshara karma method of treating piles has been described in detail in the ancient text Sushruta Samhita. The details of preparation and its herbal composition with combination are mentioned in Sushruta Samhita and Ashtanga Hridaya. And also, the superiority of kshara over sharp instruments (sastras) and accessory sharp instruments (anusastras) has been mentioned in Sushruta Samhita. The typical features and outcomes of Kshara karma in management of hemorrhoids—Athanenaiba bidhanena kutajapalasaswakarnaparibhadrakabibhitakaragbadhati lwakarkasnuhyapamargasamulaphalap atrasakha dahet, mruprasruta- bagadanyuchritani khyarena, athopasante-agnou tadbhasma.
MATERIALS AND METHODS

We developed a method at the dept. of Dravyaguna, faculty of Ayurveda method of preparation of Pratisaraneeya teekshna kshara known as “Pervaje Technique of “Ksharakarma,” is a modification of what is described in Sushruta Samhita. 10 Kg of Achyranthes aspera (Apamarga) is burnt to get 1 liter in volume of ash. The ash is mixed with six times of water to form a solution, which is filtered using a sterile cloth twenty one times. The filtrate was boiled slowly to reduce it to 2/3 of quantity, followed by addition of
100 gm of Varatika shell red hot burnt ash powder. The solution is again boiled slowly with adding of 10 gm of fine rubbed powdered Citraka (Plumbago zeylanica), up to get semisolid, brownish-colored paste; this is collected and stored in dry glass bottle.

This Alkaline preparations (Pratisharniya kshara) is indicated for externally used in skin diseases like Psoriasis, Taeniais, Vitiligo, Non-lepromatous lesion, Fistula in ano, Hemorrhoids, Tumors, Non-healing ulcer, Sinus, Exfoliative dermatitis, Mole, Non-elevated mole, Localized hyper pigmentation of skin, Nevus, and Abscess.\(^7\) In cases of worms and poisoning as well as in the seven forms of diseases which affect the cavity of the mouth and in different types of Diphtheria, external applications of alkalis act like substitutive surgical instruments.\(^10\)

**Animals:** The protocol for the study was approved by the ethical Research Committee of Institute of Medical Sciences, Banaras Hindu University of Medical Sciences, Varanasi. Non-pregnant Swiss albino mice weighing 25-30 g approx. were obtained from the Animal house dept of Anatomy All animals were maintained 8 days under the same laboratory conditions of temperature (22°C ± 3°C), relative humidity (55% ± 5%), and a 12 hours light/dark cycle (1:1) and received a nutritionally standard diet (Hindustan Liver diet) and tap water ad libitum. All mice were treated with outmost human care.

**Experimental protocol and Histology:** Animals were randomly divided into two groups, with 20 mice per group. The first group received Integra and the second group was treated with Kshara karma. Two, Four, six and eight days after the surgery, five mice from each group were euthanized, the transplant area was excised, halved, fixed and embedded in paraffin for histological procedures as described below.

**Histological procedure:** Excised tissues were fixed in 10% formalin solution for five days. All tissues were processed by using conventional histo-chemical techniques, embedded in paraffin wax and then sectioned at 6 μm thicknesses, mounted on glass slides, deparaffinized and stained with Haematoxylin–Eosin (HE). The numerical density per area (NA of inflammatory cells or blood vessels was determined in a 45 μm\(^2\) frame. Random fields of the tissue were counted for each section. Images were taken with an optic microscope (Ni, Nikon) and digital sight camera (QC color, 3C Q-Imaging). The granulation tissue was assessed as the increase in thickness (μm) measured in the largest portion of each ulcer. The analyses were carried in a blinded manner.
Statistical analysis

Values were expressed as the mean ± SEM with the indicated numbers (n) of experiments. Kolmogorov-Smirnov test was used to assess the normality. Unpaired two-tailed t-test was used to determine significance. Statistical analyses were performed with Graph Pad Prism 6 (Graph Pad Software, La Jolla, CA). Values of p < 0.05 were considered significant.

OBSERVATION AND RESULT

Table 1: Various parameters taken for dissection of rectum and results of application of test

<table>
<thead>
<tr>
<th>Parameters</th>
<th>*Control mice rectum No. of experiment(n=5)</th>
<th>**2nd day T.K. tr. mice rectum No. of experiment(n=5)</th>
<th>***4th day T.K. tr. mice rectum No. of experiment(n=5)</th>
<th>****6th day T.K. tr. mice rectum No. of experiment(n=5)</th>
<th>*****8th day T.K. tr. mice rectum No. of experiment(n=5)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean ± SEM in mm.</td>
<td>Musclelayer-2.05±0.003 Mucous layer-3.25±0.004</td>
<td>Musclelayer-2.45±0.004 Mucous layer-3.65±0.005</td>
<td>Musclelayer-2.25±0.003 Mucous layer-3.45±0.004</td>
<td>Musclelayer-1.95±0.002 Mucous layer-3.05±0.003</td>
<td>Musclelayer-1.75±0.001 Mucous layer-2.95±0.002</td>
</tr>
<tr>
<td>Kolmogorov-Smirnov test(α)</td>
<td>Highly sig.(0.03) 0.1/1.22</td>
<td>Highly sig.(0.02) 0.05/1.36</td>
<td>Highly sig.(0.03) 0.025/1.48</td>
<td>Highly sig.(0.02) 0.005/1.73</td>
<td>Highly sig.(0.01) 0.001/1.95</td>
</tr>
<tr>
<td>Unpaired 2-tailed t test</td>
<td>Control vs 2nd, 4th, 6th &amp; 8th = Highly sig.(0.03)</td>
<td>2nd vs Control, 4th, 6th &amp; 8th = Highly sig.(0.02)</td>
<td>4th vs Control, 2nd, 6th &amp; 8th = Highly sig.(0.03)</td>
<td>6th vs Control, 2nd, 4th &amp; 8th = Highly sig.(0.02)</td>
<td>8th vs Control, 2nd, 6th &amp; 8th = Highly sig.(0.01)</td>
</tr>
<tr>
<td>P-value</td>
<td>0.03</td>
<td>0.02</td>
<td>0.03</td>
<td>0.02</td>
<td>0.01</td>
</tr>
</tbody>
</table>

Table represents *Control mice rectum, **2nd day T.K. tr. mice rectum, ***4th day T.K. tr. mice rectum, ****6th day T.K. tr. mice rectum, *****8th day T.K. tr. mice rectum

The 2nd day dissected non pregnant mice rectum histological findings showed large hemorrhagic scab with gross inflammation of peri circumferential zone with total necrosis of tissue, adequate tissue sloughs were liberated. The H&E stained histological zone showed large slough area with foul smelled purulent discharge. Adequate neutrophil infiltration was a common sign and symptom executed, gross destruction and distortion of auto architecture of mucosa and muscle layer of rectum was seen as a rectal profile with slough area showed eosinophilic mass infiltration with the scab. The 4th day dissected non pregnant mice rectum histological findings showed scattered and adequate infiltration of neutrophils at the base of the slough area and ulcer in comparison to the control one. The 6th day dissected non pregnant mice rectum histological findings showed infiltration of lymphocyte with progression of reepithelisation and healing of the same. The 8th day dissected non pregnant
mice rectum histological findings showed small stunted villi’s in comparison to the control with multiple focal appearance of the newly developed fibroblasts of the same at the surrounding and the base of the wound zone.

Multiple measurements were taken from breadth of different layer of dissected rectums of control, 2\textsuperscript{nd}, 4\textsuperscript{th}, 6\textsuperscript{th} and 8\textsuperscript{th} day dissected teekhna kshara through anal treated Swiss albino non pregnant mice. Values were expressed as the mean ± SEM with the indicated numbers (n) of experiments. Kolmogorov-Smirnov test was used to assess the normality. Unpaired two-tailed t-test was used to determine significance. Values of p < 0.05 were considered significant. Given below in tabulated form.

\[\text{̃-Histological panoramic view of normal rectum in 40x,}\ \text{́-T.S. of rectum of 2}^{\text{nd}}\ \text{day dissected teekhna kshara treated showing muscle and mucous layer}\ \text{verbs-} \text{Histological panoramic view of 2}^{\text{nd}}\ \text{day teekhna kshara treated muscle layer of rectum}, \text{́-Histological panoramic 2}^{\text{nd}}\ \text{view of 2}^{\text{nd}}\ \text{day teekhna kshara treated muscle layer of rectum} \]

“Fig.1”: 2\textsuperscript{nd} day histological findings of dissected rectum of teekhna kshara treated mice.

Anal mucosa of 4\textsuperscript{th} day euthanized group of subsequent 5 mice showed more increase of inflammation, more liberation of slough tissue with more aggravation of foul smelling of affected area grossly, and some part of tissues were found sloughed with purulent discharge from the area of anus with stacking of stool on side wall of anal mucosa, anal opening found constricted with overloading of faecal matter just inside grossly histologically it showed more increase of break and dysmorphism of anal mucosa and sub mucosa with continuous increase of hyperemic blood vessels where multiple hemorrhagic patches found scattered on histological fields also found increased . Sloughing off local anal mucosa and sub mucosa with epithelium found in clumping manner more in comparison to 2\textsuperscript{nd} day euthanize mice.
group and also found segregated from normal tissue and especially slough is found at the base of the ulcer. Raised area of hyperemic feature found more with more increased of contour and thickness compare to 2nd day euthanize mice group, Neutrophil cells infiltration was found in the secondary stage but the intensity and number counted of this group was found lesser from 2nd day euthanize group.

1. Histological panoramic view of normal rectum in 40x
2. Histological panoramic view of 4th day dissected teekhna kshara treated muscle layer of rectum in 40x
3. Histological panoramic view of 4th day dissected teekhna kshara treated muscle and mucous layer of rectum in 40x

“Fig.2”: 4th day histological findings of dissected rectum of teekhna kshara treated mice

Anal mucosa of 6th day euthanized group of subsequent 5 mice showed beginning of gradual reduce of inflammation, beginning of stoppage activity of liberation of slough tissue with foul smelling of affected area was found gradually reduced grossly with visualize reduction of purulent discharge and sloughing from the area of anus and beginning of granulation with fibrosis of affected with reepithelisation area grossly histologically it showed reduction of break and dysmorphology of anal mucosa and sub mucosa with natural repair and healing process, neutrophil infiltration, hyperemic blood vessels found reduce of hypertrophy and gradually stoppage of blood flow to affected area where, hemorrhagic patches were found less scattered on histological fields in comparison to 2nd day and 4th day euthanized mice group. Sloughing action gradually found stopped in comparison to 2nd day and 4th day euthanize mice group, contour and thickness of local tissue found reduced compare to 2nd day and 4th day euthanize mice group. The sub mucosal lining shows gross infiltration of neutrophils at the base of the slough area. Neutrophil cells infiltration was also found in the tertiary stage with intensity and number counted of this group was found lesser from 2nd and 4th day euthanize group.
Histological panoramic view of normal rectum in 40x

Histological panoramic view of 6th day dissected teekhna kshara treated muscle and mucous layer of rectum in 40x

“Fig.3”: 6th day histological findings of dissected rectum of teekhna kshara treated mice

Anal mucosa of 8th day euthanized group of subsequent 5 mice showed complete stop of inflammation, complete stop of liberation of slough tissue with foul smelling of affected area, complete stop of purulent discharge and sloughing from the area of anus and highest percentage of granulation with fibrosis of affected area grossly histologically it showed same intensity of break and dysmorphology of anal mucosa and sub mucosa with natural repair and healing process as seen in 6th day euthanized mice group, hyperemic blood vessels found complete stop of inflammation with normal contour and thickness and complete stop of blood flow to affected area where no or very few hemorrhagic patches found on histological fields in comparison to 2nd day, 4th day and 6th day euthanized mice group. Neutrophil cells infiltration was found in the quaternary stage with intensity and number counted of this group was found lesser from 2nd, 4th and 6th day euthanize group. Small stunted villi’s is also found in comparison to control with multiple appearance of newly developed fibroblast.

“Fig.4”: 8th day histological findings of dissected rectum of teekhna kshara treated mice

Histological panoramic view of normal rectum in 40x

Histological panoramic view of 8th day dissected teekhna kshara treated muscle and mucous layer of rectum in 40x
DISCUSSION
Teekhna kshara after topical application on testing over the anal mucosa of transitional, pectinate and anal zone in animals causes violate bluish discoloration of the area, hyperemic features and forceful flow of surrounding venous blood to the topically applied area which gradually progresses in due course of time with sharp aggravation of pain is clearly visualize in the experimental animal where the victim is found shouting agonizingly and running in a hurry here and there inside the animal cage immediate after application. The pain, hyperemic features and bluish discoloration were slowly subsided immediately after secondary application of lime juice with a gauge rolled piece of thin coca nut broom stick on the primary kshara applied area of anal mucosa.

Anal mucosa of 2\textsuperscript{nd} day euthanized group initial 5 mice after application of teekhna kshara showed excess inflammation, beginning of liberation of slough tissue with aggravation of foul smelling of affected area grossly, histologically it showed multiple break and dysmorphology of anal mucosa and sub mucosa with hyperemic blood vessels where multiple hemorrhagic patches found scattered on histological fields. It also showed haemorrhagic mass with large haemorrhagic scab. Tissue slough was predominantly found in different areas of histological section with large areas of neutrophil infiltration. Distortion of auto architecture of mucosa of rectum was seen as major findings. Slough areas showed eosinophilic mass with the scab. Beginning of the sloughing off local anal mucosa and sub mucosa with epithelium found in clumping manner which is either partially or fully segregated from normal tissue, Raised area of hyperemic feature found which is clearly differentiated from normal area with increased contour and thickness compare to control animals, Neutrophil cells infiltration was found in the beginning stage but the intensity and number counted of this group was found higher from 4\textsuperscript{th}, 6\textsuperscript{th} and 8\textsuperscript{th} day euthanize group where the control group showed minimal invasion of neutrophil cells.

CONCLUSION
The present histological and gross study on kshara karma in the aspect of treatment and management of hemorrhoids concludes that teekhna kshara causes gradual increase of sloughing, discoloration, blood flow with hyperemicity and inflammation of the local tissue on topically applied area in the beginning stage with aggravation of pain whereas in the delayed stage it causes healing with complete granulation and fibrosis of the same.
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REFERENCES

1. M.N. Ch. 5; 63.