COMPARATIVE STUDY OF RASAPARPATI- AN AYURVEDIC FORMULATION ON INTESTINAL MOTILITY AND ITS AMELIORATIVE EFFECTS ON MUCOSAL DAMAGE IN RAT MODEL OF ULCERATIVE COLITIS

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ABSTRACT

Background: Ayurvedic classics claim the usefulness of Rasaparpati in Grahani diseaeses. As per modern view, Grahani is equated with malabsorption syndrome, disturbed intestinal motility, chronic colitis etc. Various methods described for Shodhana (purification and/or detoxification) of Parada (mercury) used as one of the ingredients in the preparation Rasaparpati. Therefore, in the present study three different formulations of Rasaparpati prepared by SamanyaSodhita, Astasamskarita and AnuvasitaParada and evaluated for its efficacy on intestinal motility and ameliorative effects in modulating the extent and severity of experimental ulcerative colitis in rats. Materials & methods: Effect of Rasaparpati samples on intestinal motility was checked through latency of onset of kaolin expulsion through GI tract in mice. Test drugs treated mice received respective sample of Rasaparpati (32.5 mg/kg, po). The anti-colitis activity was assessed through acetic acid-induced experimental colitis in Charle’s foster rats. Test drugs were administered for 7 consecutive days to respective groups in dose of 22.5 mg/kg body weight of rats. Colitis was induced by intra-colonic instillation of 5 ml/kg of 4% (v/v) acetic acid in rats. The inflammatory response was assessed by effect on severity of ulceration and histopathological study. Result: Rasaparpati prepared by
AistasamskaritaParada produced increase in intestinal motility and fecal output in mice. Rasaparpati prepared by Astasamskarita and AnuvasitaParada administrated groups produced an apparent decrease in the area and severity of ulceration in experimental colitis in rats. However, it was found to be statistically non-significant. Rasaparpati prepared by AstasamskaritaParada administered group produced better result in acetic acid-induced ulcerative colitis. **Conclusion:** These findings reveal the effects of Rasaparpati prepared by AstasamskaritaParada in intestinal motility and down regulation of inflammatory response in acetic acid-induced colitis in rats.

**KEYWORDS:** Grahani, Intestinal motility, Parada, Rasaparpati, Ulcerative colitis.

**INTRODUCTION**

The ancient Ayurvedic texts like CarakaSamhita have defined the concept of shodhana. It says that karana (Processing) is the refinement of the natural products which means imparting other properties. These properties are infused by eight ways, one of them is sauca (Cleansing).[1] The concept of shodhana treatment was highly accepted by the pioneers of Rasashastra (8th Century A.D.) especially for the purification of herbo-mineral drugs. The purification treatments were basically meant to reduce the toxicity level to a body-sustainable limit. In ancient Rasasastra texts, different types of Parpaties have been mentioned according to their principle drug, ingredients, bhavana drugs and so on. Presence of Parada in Parpatiis known as Rasaparpati. Various methods described for Shodhana (purification and/or detoxification) of parada (mercury) used as one of the ingredients in the preparation Rasaparpati. Therefore, in the present study, three different formulations of Rasaparpati prepared by SamanyaSodhita, Astasamskarita and AnuvasitaParada were used for experimental study.

Rasaparpati is an inorganic preparation, containing mercury and sulphur and some trace organic elements. Ayurvedic classics claim the usefulness of Rasaparpati in Grahani diseases. Caraka has mentioned the pathological condition of Grahani that occur due to vitiated Agni. Any sort of vitiation that occur in Agni will lead the Grahani to excrete Vidagdha Ahara either in the form of undigested or digested stools[2] which is termed as Grahani Dosha. As per modern view, Grahani is equated with malabsorption syndrome, disturbed intestinal motility, chronic colitis etc. Ulcerative colitis is one of the inflammatory bowel diseases (IBD), which may occur due to different etiological factors sometimes simulating the signs and symptoms of Grahani disease. The etiology of IBD remains
unknown, although it is believed that deregulated immune response along with several intra and extra multi-factorial intestinal manifestations including environmental, genetic and auto immune phenomena influences both the initiation and progression of IBD.\[3\]

On the basis of therapeutic efficacy of Rasaparpati, experimental models were planned to evaluate the effect of test drugs. In ancient Ayurvedic classics too, an emphasis has been placed on animal experimentation prior to its administration to human beings. As Acharya Susruta states the importance of animal experimentation in literature.\[4\] Thus, to justify the use of method for shodhita parade in Rasaparpati, animal experimentation has been carried out with the effects of trial drugs - Rasaparpati prepared from samanyasodhita parada, astasamskarita parada & anuvasita paradaon intestinal motility in albino mice and acetic acid-induced ulcerative colitis in albino rats.

MATERIALANDMETHOD

Animal
Swiss albino mice (25±5 g) and Charles foster albino rats (200±30) of either sex were procured from the Animal House attached to Pharmacology Laboratory, IPGT and RA, Gujarat Ayurved University, Jamnagar, Gujarat. They were housed in standard husbandry conditions and fed with Amrut brand rat pellet feed and drinking water given ad libitum. The animals were acclimatized for at least one week before commencement of the experiment.

Drugs
Fine powder (200 mesh) of Rasaparpati\[5\] samples prepared by SamanyaSodhita Parada\[6\] (Sample A), Astasamsakarita Parada\[7\] (Sample B) and Anuvasita Parada\[8\] (Sample C).

Dose
The normal adult dose of Rasaparpati is 125-250 mg/day. Hence, the dose for the mice and rat was calculated on the basis of body surface area ratio by referring the standard table of Paget and Barnes.\[9\] Based on calculation, the oral dose of Rasaparpati for mice was fixed as 32.5 mg/kg and rat was 22.5 mg/kg. The test drug was suspended in distilled water with suitable concentration depending up on body weight and administered orally with the help of oral catheter.
Experimental design

Effects of test drugs on intestinal motility in mice

The selected mice were divided into four groups of eight each comprising four male and four females. Group (I) served as control and received distilled water (10 ml/kg, po) while Group (II), (III) and (IV) kept as drug treated groups received Rasaparpati samples (32.5 mg/kg, po) prepared by SamanyaSodhita Parada (Sample A), Astasamsakarita Parada (Sample B) and AnuvasitaParada (Sample C) respectively to overnight fasted animals. The effect of the formulation on intestinal transit time was carried out based on previous study.[10] one hour after test drug administration to respective groups, 0.1 ml of 40% w/v Kaolin suspension was administered to all the animals with the help of oral catheter. The animals were placed in a transparent arena and were carefully observed for the beginning of the kaolin expulsion which begins in the form of white colored fecal pellets. The time for onset of kaolin expulsion was noted as index of intestinal motility in mice.

Effects of test drugs on experimental-induced ulcerative colitis

Charle’s Fosterrats weighing between 200±30g were divided into 4 groups, each consisting of six rats. Group (I) served as control and received distilled water (10 ml/kg, po) while Group (II), (III) and (IV) kept as drug treated groups received Rasaparpati samples (22.5 mg/kg, po) prepared by SamanyaSodhitaParada(Sample A), AstasamsakaritaParada(Sample B) and AnuvasitaParada(Sample C) respectively. The drugs were administered for 7 consecutive days in the morning hours. The method of acetic acid-induced colitis was adapted from that originally described by previous author with slight modification as per experimental need.[11] Rats were isolated and kept in metabolic cage for overnight fasting with access to water ad libitum on 7th day. On 8th day, one hours after test drugs administration, under light anesthesia an enema of 5 ml/kg of 4% (v/v) acetic acid was infused in to lumen of colon by using polyethylene tube (2 mm in diameter) which was inserted through rectum in to the colon to a distance of 8 cm proximal to the anus verge. Again, the rats were isolated and kept in metabolic cage for next 24 hours and afterwards animals were scarified.

For microscopic analysis, rapidly colon was excised, opened along its antimesenteric border; gently rinsed of its luminal contents with saline solution and immediately examined macroscopically with naked eyes and also with the aid of a magnifying lens. Macroscopic analysis on severity of ulceration was assessed as described by Morries et al. (1989).[12]
Percentage of area of ulceration was calculated by recording the total area of colon and percentage of it affected by ulceration.

**Statistical analysis**
The data were expressed as mean ± standard error of mean (SEM). The significance of differences among the groups was assessed using Students ‘t’ test for unpaired data. P<0.05 was considered as statistically significant and values were interpreted accordingly.

**RESULTS AND DISCUSSION**
In this experiment, to assess the action of three dosage forms of Rasaparpation the intestinal motility, latency of onset of kaolin expulsion in fecal matter was selected as a parameter. Effects of test drugs on intestinal transit time of kaolin have been provided in Table1. Rasaparpati prepared by AstasamskaritaParada produced decrease intestinal transit time of kaolin i.e. increase in intestinal motility and fecal output in mice in comparison to control group. However, the observed changes were found to be statistically non-significant. Rasaparpati prepared by SamanyaSodhita and AnuvasitaParada did not produce any alteration in intestinal transit time in comparison to control group. In the classical literature, it has been clearly mentioned that Virechana can act as a curative, preventive, and health promotive measure.\(^{[13]}\)

As, it was difficult to assess in vivo movement of the drug, it was thought useful to administer a marker, which causes color change of fecal matter and doesn’t alter the effect of drug. In the present study, Rasaparpati prepared by AstasamskaritaParada produced increase in intestinal motility and fecal output in mice. The mechanism of observed effect may be due to the interference with local stimulant effect on motility or acceleration of gastric emptying. The neural regulation of gastric motility involves stimulation by cholinergic neurons inhibition by adrenergic neurons. Antagonist of D2 and 5-HT3 receptors as well as agonists of 5-HT4 receptors can stimulate gastric motility.\(^{[14,15]}\) Some of the drugs increase the motility of intestine by modifying the fluid dynamics of the mucosal wall and may cause fluid accumulation in lumen. In the present study, Rasaparpati prepared by AstasamskaritaParadadid not affect the consistency of the fecal matter hence; shortening of duration required for the expulsion of Kaolin may be due to increased intestinal motility by local stimulant effect on motility or acceleration of gastric emptying.
Induction of colitis by acetic acid in rats is one of the standardized and well established commonly used methods to produce an experimental model of inflammatory bowel disease with some resemblance to human acute intestinal inflammation. The rectal administration of acetic acid produced severe macroscopic mucosal inflammation, ulceration and hemorrhagic lesions in the colon of rats (Table 2). Rasaparpati prepared by Astasamskarita and Anuvasita Parada administrated groups produced an apparent decrease in the area and severity of ulceration in experimental colitis in rats. However, it was found to be statistically non-significant. Ulcerative colitis is associated with high-level activity of myeloperoxidase activity. Further acetic acid also enhanced cellular lipid peroxidation. Drugs which decrease generation of reactive oxygen metabolites like super oxide and singlet organ or those which enhance the activity of endogenous anti-oxidant system are likely to be effective in decreasing the severity of acetic acid induced colitis. It is possible that the test samples may possess similar mechanism of action, which may be responsible for the observed moderate anti-colitis activity. Based on both histopathological and pharmacological study it can be suggested that Rasaparpati prepared by Astasamskarita Parada administered group produced better result in acetic acid-induced ulcerative colitis.

In Ayurvedic literature pertaining to Rasasastra, certain specific processing techniques have been described which are supposed to be helpful in converting raw drugs into a finished product of desired therapeutic efficacy. Rasaparpati is such an example in which Saraka property of Kajjali gets converted into Grahi property. Gandhaka is having Katuvipaka, UshaVirya, Dipana, Pacana and Yogavahita properties. It combines with Parada, which is having Yogavahitaguna. On trituration of these two drugs together, the Kajjali form which is having Sara and GuruGuna will be affected while, preparing Parpati, Sara and GuruGuna of Kajjali along with the heating and synergetic effect (Samskarasyanuvartanam) of Ghṛta, KadaliPatra and Gomaya transform into Laghu and GrahiGuna. These Grahi and LaghuGunas digest Ama and restore the Agni, thereby eradicating the RogaGrahani.

Table 1: Effect of Rasaparpati samples on intestinal transit time in mice.

<table>
<thead>
<tr>
<th>Groups</th>
<th>Dose (mg/kg)</th>
<th>Kaolin pellet expulsion time (mins.)</th>
<th>% change</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>-</td>
<td>330.63 ± 33.88</td>
<td>-</td>
</tr>
<tr>
<td>Rasaparpati A</td>
<td>32.5</td>
<td>331.00 ± 39.86</td>
<td>0.11 ↑</td>
</tr>
<tr>
<td>Rasaparpati B</td>
<td>32.5</td>
<td>257.50 ± 32.72</td>
<td>22.12 ↓</td>
</tr>
<tr>
<td>Rasaparpati C</td>
<td>32.5</td>
<td>339.00 ± 42.14</td>
<td>2.53 ↑</td>
</tr>
</tbody>
</table>

Data: Mean±SEM; ↓- Decrease, ↑- Increase
Table 2: Effect of *Rasaparpati* samples on severity and percentage area of ulceration in acetic acid induced colitis in rats.

<table>
<thead>
<tr>
<th>Groups</th>
<th>Dose (mg/kg)</th>
<th>Severity of ulcers</th>
<th>% area of ulceration</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>-</td>
<td>3.4 ± 0.6</td>
<td>59.40 ± 10.30</td>
</tr>
<tr>
<td><em>Rasaparpati</em> A</td>
<td>22.5</td>
<td>3.2 ± 0.4 ↓</td>
<td>59.80 ± 09.30 ↑</td>
</tr>
<tr>
<td><em>Rasaparpati</em> B</td>
<td>22.5</td>
<td>2.4 ± 0.2 ↓</td>
<td>52.70 ± 4.50 ↓</td>
</tr>
<tr>
<td><em>Rasaparpati</em> C</td>
<td>22.5</td>
<td>3.8 ± 0.2 ↑</td>
<td>45.08 ± 5.44 ↓</td>
</tr>
</tbody>
</table>

Data: Mean±SEM; ↓- Decrease, ↑- Increase

CONCLUSION

From the present study it is concluded that *Rasaparpati* prepared by *AstasamskaritaParada* having better effects than *Rasaparpati* prepared by *SamanyaSodhita* and *AnuvasitaParada* on intestinal motility in mice and down regulation of inflammatory response in acetic acid-induced colitis in rats.

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