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Research Article

Evaluation of *Rasanjanadi Churna* in Acetic acid induced Ulcerative Colitis in Rats

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Abstract

Introduction: Ulcerative Colitis is an idiopathic form of acute and chronic ulcero-inflammatory colitis affecting chiefly the mucosa and submucosa of the rectum and descending colon. Drug resistance, drug dependency and side effects of those drugs are seen in long term treatment according to modern medicine. The signs and symptoms of Ulcerative Colitis show much resemblance with the symptoms of *Raktatisaar*. Therefore, an *Ayurvedic* herbal formulation namely *Rasanjanadi Churna* mentioned in *Yogratnakar Atisaar Adhyaya Raktatisaar Adhikaar* is chosen for study in Ulcerative Colitis. Objective- To evaluate the efficacy of *Rasanjanadi Churna* in Acetic acid induced Ulcerative Colitis in Albino Wistar Rats. Materials and Method- 24 Rats were randomly selected into 4 groups namely Disease Control, Standard, Test and Vehicle. All groups were given drugs from 1 to 7 days and colitis was induced on Day 4 by Intrarectal administration of 500 µL acetic acid (4%). On Day 8, all animals were sacrificed and parameters were studied. Observation and Result- Assessment criteria were Body Weight, Clinical Sign and Symptom, Assessment of Stool, Macroscopic assessment of Colon, Haematological parameter, Biomarker oxidative Assay (Catalase, Superoxide dismutase and Malonaldehyde), Serum IL-6, Serum IgA parameter, Microscopic assessment of Colon, Colon weight to length ratio. Conclusion- The findings of the Pre- Clinical study indicate that *Rasanjanadi Churna* showed positive results against the damaging effect of acetic acid induced colitis in Wistar rats. *Rasanjanadi Churna* showed equivalent results in all the parameters when compared with Standard drug Sulphasalazine.

Keywords: *Rasanjanadi Churna,* Preclinical Study, Ulcerative Colitis, Acetic acid induced Ulcerative Colitis.

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Introduction

The Incidence of Ulcerative Colitis in India is 6.02/100,000 (1). Ulcerative Colitis is an idiopathic form of acute and chronic ulcero-inflammatory colitis affecting chiefly the mucosa and submucosa of the rectum and descending colon, though sometimes it may involve the entire length of the large bowel (2). The cardinal symptoms are rectal bleeding with passage of mucus and bloody diarrhoea. In severe cases, anorexia, malaise, weight loss and abdominal pain occur (3). Drug resistance, drug dependency and side effects of those drugs are seen in long term treatment according to modern medicine (4). The signs and symptoms of Ulcerative Colitis show much resemblance with the symptoms of *Raktatisaar* (5). Therefore, an *Ayurvedic* herbal formulation namely *Rasanjanadi Churna* (6) mentioned in *Yogratnakar Atisaar Adhyaya Raktatisaar Adhikaar* is chosen for study in Ulcerative Colitis. Preclinical studies carried on some contents of *Rasanjanadi Churna* have shown antidiarrheal (7) (8), anti-inflammatory (9) or wound healing activity (10) (11). Therefore, there is a need to evaluate the combined effect of all contents of *Rasanjanadi Churna* in Preclinical Study.

Objective

To evaluate the efficacy of *Rasanjanadi Churna* in Acetic acid induced Ulcerative Colitis in Albino Wistar Rats.

Materials and Method

1. Induction Drug- Acetic Acid

2. Vehicle- Honey was obtained from Standard Company.

3. Standard Drug- Sulfasalazine Gastro- Resistant tablets IP 1g

4. Test Drug- *Rasanjanadi Churna* was prepared according to the classical text *Yogratnakar- Atisaar Adhyaya Raktatisaar Adhikaar* (6)

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Table 1: Ingredients of *Rasanjanadi Churna* (6)

Sr. no.	Ingredients	Latin name	Family	Part used
1	*Daruharidra*	*Berberis aristata DC.*	*Berberidaceae*	Stem
2	*Ativisha*	*Aconitum heterophyllum Wall. ex. Royle*	*Ranunculaceae*	Root
3	*Kutaj phala (Indrayav)*	*Holarrhena antidysentrica(Wall)*	*Apocynaceae*	Seeds
4	*Kutaj Twak*	*Holarrhena antidysentrica(Wall)*	*Apocynaceae*	Stem bark
5	*Dhataki*	*Woodfordia fructicosa (Linn.) Kurz.*	*Lythraceae*	Flower
6	*Shrungaber (Shunthi)*	*Zingiber officinale Rosc.*	*Zingiberaceae*	Rhizome

All the raw materials were procured from local market and authenticated from Quality Control Laboratory. Goat milk was obtained from local Goat milk farm. The raw materials were subjected to physico-chemical Analysis before using them in formulation.

Preparation of *Rasanjanadi Churna* was carried out in two steps:

Preparation of *Rasanjan* (28)

One part of *Daruharidra Twak Bharad* and 16 parts of water were soaked overnight. Next day it was heated on medium heat and reduced to 1/8th. The *Kwath* was filtered with cotton cloth. *Kwath* was again reduced till it obtained syrup like consistency. Then warm ¼th goat milk was gradually added and stirred continuously. The mixture was heated and continuously stirred till it attained consistency of *Rasanjan.* After removing from heat, *Rasanjan* was dried completely at 32°C in Hot Air oven. The dried *Rasanjan* was made into powder by mixer. Then powdered *Rasanjan* was sifted by Sieve No.85.

Preparation of *Rasanjanadi Churna* (6) (29)

Dried *Ativisha* root, Dried *Indrayav* seeds, Dried *Kutaj* bark, Dried *Shunthi* rhizome were individually pounded in *Udukhal Yantra* (Mortar and pestle) and grinded in Mixer. Then individual powders were sifted with Sieve No. 85 and *Churna* were obtained. Dried *Dhataki* flowers were grinded in Mixer. Then the powder was sifted with Sieve No. 85 and *Dhataki Churna* was obtained. All the above *Churna* namely *Ativisha Churna, Indrayav Churna, Kutaj Churna, Dhataki Churna* and *Shunthi Churna* and previously prepared powdered *Rasanjan*, were taken in equal proportion and uniformly mixed in a mixer. The entire mixture was again sifted through Sieve No. 85 and *Rasanjanadi Churna* was obtained.

Preparation of Animal model

24 Albino Male Wistar Rats weighing 200- 250 g were selected for study. Identification mark was given to animals and cages. Six rats were placed in each cage with clean paddy husk. The rats were acclimatized at test environment for 7 days prior to dosing. Room temperature maintained between 22 +3°C, relative humidity 55 + 5 % and 12-hours light and 12 hours' dark cycle was maintained.

Inclusion Criteria: Either gender

Exclusion Criteria: Previously used for experiment, Pregnant

Diet: Pelleted feed supplied by Nutrivet Pvt. Ltd. ad libitum during the study.

Water: RO filtered water was provided ad libitum.

Animal Welfare: All procedures such as Housing, dosing, sacrifice, rehabilitation was done in accordance with the standard operating procedures and the guidelines provided by the Committee for the Purpose of Control and Supervision of Experiments on Animals (CCSEA) as published in The Gazette of India, December 15, 1998 and Biological evaluation of medical devices- Part 2: Animal welfare requirements. Study has been approved in Institutional Animal Ethics Committee meeting.

Dose Calculation

1. *Rasanjanadi Churna*

A) Human dose- 1 *karsha* (12 gm approx.) daily (Sharangdhar Samhita and Yogratnakar)

B) Animal dose- 216mg/200g daily (1.080g/kg) As per Paget and Barnes formula

2. Sulfasalazine

A) Human dose - 3-4g/day

B) Animal dose- 500mg/kg

3. Vehicle- *Tandulodak* and Honey

A) *Tandulodak*- a) Human dose- 4 *karsha* (48 ml approx.) daily

b) Animal dose- 0.864ml/200g daily (4.32ml/kg)

B) Honey - a) Human dose- 2 *karsha* (24ml approx.) daily

b) Animal dose- 0.432ml/200g daily (2.16ml/kg)

4. Anesthesia

75 + 10 mg/kg of Ketamine & Xylazine was given intraperitoneally (i.p) for anesthesia at the time of bleed

Procedure

Six male Wistar rats (8-12 weeks old, weighing 200-250g) in each group were used for this study. Rats were randomly selected into 4 groups.

Table 2: Groups

Group	Name of Group	Drug	Dose	No. of Animals (Wistar Rats)
1	Disease	Acetic acid	500 µL	6
2	Standard	Sulfasalazine	500 mg/kg	6
3	Test	*Rasanjanadi Churna*+ *Tandulodak*+ Honey	1080 mg/kg + 4.32mL/kg + 2.16 mL/kg	6
4	Vehicle	*Tandulodak*+ Honey	4.32mL/kg + 2.16 mL/kg	6

- Test Group was given *Rasanjanadi Churna* with *Tandulodak* and Honey, Vehicle Group was given *Tandulodak* and Honey, Standard Group was given Sulfasalazine for 7 days and Disease Control Group was not given drug orally for 7 days.
- On day 4, colitis was induced in all groups by Intrarectal administration of 500 µL of acetic acid (4%).

- Body weights of the animals were recorded daily.
- Anesthesia was provided to animals after completion of treatment on 8th day and collection of blood was done for hematological, IL-6 & IGA parameters.
- At 8th day all the animals were sacrificed by exposure of Carbon dioxide + Chloroform. Organ weights were recorded after sacrifice. Colon was collected for histopathological examination.
- Part of the colon was processed for the estimation; Colons were homogenized using on ice-cold Tris-HCl buffer (0.01 M, pH 7.4) to give a 10% homogenate were used for assays of Superoxide dismutase(SOD), Malondialdehyde (MDA) Level and Catalase (CAT) activity in the colons.

Assessment Criteria

A. Body Weight Records - Body weight of each animal was recorded before the treatment, weekly, and at the end of the study.

B. Clinical Sign and Symptom - All animals were observed for the signs such as change in skin, fur, eyes, mucous membranes, respiratory, circulatory, and autonomic and central nervous systems, somatomotor activity, behavior pattern. Attention was directed to the observations of tremors, convulsions, salivation, diarrhea, lethargy, sleep and coma.

C. Assessment of Stool - Consistency of Stool and blood in Stool of Rats were be examined from day 0 to day 7.

D. Macroscopic Assessment of Colonic Damage - Macroscopically visible injuries such as thickening, shortening, hyperemia, and necrosis were blindly scored from 0-100% based on increasing order of severity.

E. Hematological parameter - After 7 days of treatment, blood samples were collected via retro-orbital puncture under light ether anesthesia. Blood was put in tubes containing a substance EDTA (15-20 IU per ml of blood) for checking hematological parameters like total number of WBCs, RBCs, Platelets and hemoglobin (Hb) % were measured by using automated Hematology System.

F. Assessment of Antioxidant Enzyme in the Colon - Part of the colon were processed for the estimation; Colons were homogenized using on ice-cold Tris-HCl buffer (0.01 M, pH 7.4) to give a 10% homogenate which were be used for assays of Superoxide dismutase (SOD), Malondialdehyde (MDA) Level and Catalase activity in the colons.

G. Assessment of Assay of Serum IL-6 & IGA -Serum samples were used for analysis by Standard Elisa Kits.

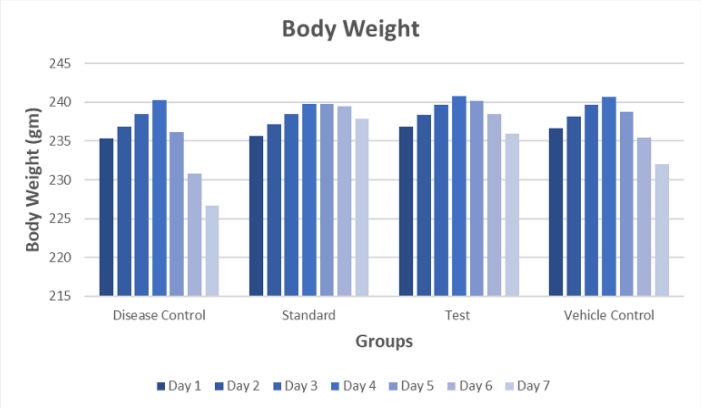
H. Microscopic Assessment of Colonic Damage - Colon segments were fixed in 10% formaldehyde and embedded in paraffin wax. For histological examination 5µm sections were cut using a microtome and stained with hematoxylin and eosin (H&E). Microscopic changes such as necrosis, fibrosis, epithelial damage, ulceration, infiltration and submucosal abscesses were scored.

I. Assessment of Colon Weight-To-Length Ratio - Rats were dissected and their colon were extirpated, opened longitudinally, and rinsed gently under saline forced with injection to remove feces. The excised colons were placed on white sheet. Each colon was weighted and length was measured to determine Weight-to-Length (mg/cm) ratio. Extirpated colons were stored at -20°C.

Observation and Result

Effect on body weight -Body weight changes were observed on weekly basis from acclimatization day to day 7 in the experimental rats.

Graph 1: Body Weight



Clinical Sign and Symptoms

Behaviour pattern was found abnormal as animals were weak and lethargic. Disease control Group showed full diarrhoea with blood and Vehicle control group showed mild diarrhoea. 3 animals were found dead in disease control group and 1 animal was found dead in vehicle control group.

Assessment of Stool

Rats were scored daily for 8 days based on stool formation (bloody or non-bloody).

Table 3: Stool Grading

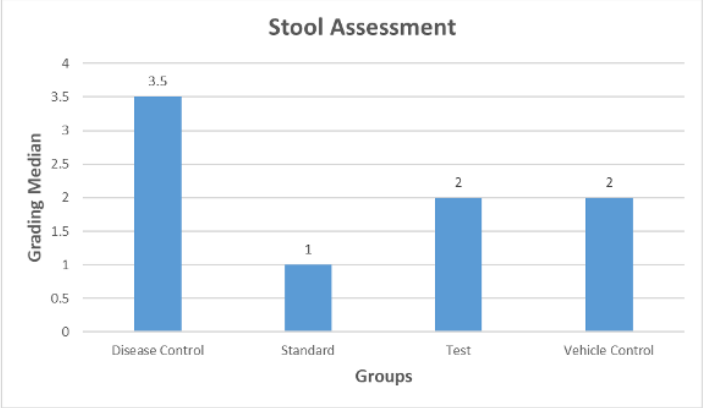
Grade	Observation
0	Normal stool consistency and dryness
1	Wet stools
2	Pasty stools
3	Mucus Stools
4	Blood stools

Table 4: Stool Assessment

Animal marking	Disease Control Group	Standard Group	Test Group	Vehicle Control Group
H	4	1	2	3
B	3	2	2	2
T	3	2	1	2
HB	4	1	1	2
BT	4	1	2	3
W	2	1	2	1
Median	**3.5**	**1**	**2**	**2**
Interquartile range	**(2.75 – 4)**	**(1 – 2)**	**(1 – 2)**	**(1.75 – 3)**
(Animal Marking on H= Head, B= Body, T= Tail, HB= Head and Body, BT= Body and Tail, W= No marking)				

A Kruskal Wallis test revealed a statistically significant difference in stool assessment across all the four groups (p = 0.004)

Graph 2: Stool Assessment



Macroscopic Assessment of Colonic Damage

Table 5: Macroscopic Grading

Grade	Observation
0	No ulcer or inflammation
1	Mucosal erythema
2	Mild mucosal edema
3	Slight bleeding or small erosions
4	Moderate edema, bleeding ulcers or erosions
5	Severe ulceration, erosions, edema and tissue necrosis

Table 6: Macroscopic Scoring

Animal marking	Disease Control	Standard	Test	Vehicle Control
H	3	1	3	3
B	4	2	1	2
T	3	2	2	2
HB	3	1	1	3
BT	3	1	2	3
W	3	2	1	2
Median	**3**	**1.5**	**1.5**	**2.5**
Interquartile range	**(3 – 3.25)**	**(1 – 2)**	**(1 – 2.25)**	**(2 – 3)**

(Animal Marking on H= Head, B= Body, T= Tail, HB= Head and Body, BT= Body and Tail, W= No marking)

Macroscopic scores across all the four groups (P= 0.003)

Graph 3: Macroscopic Score

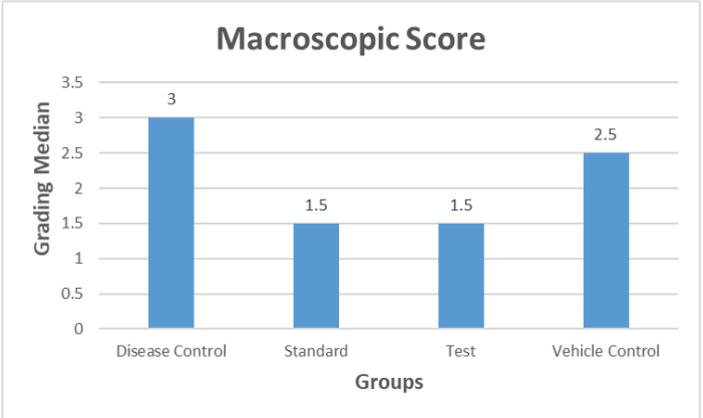
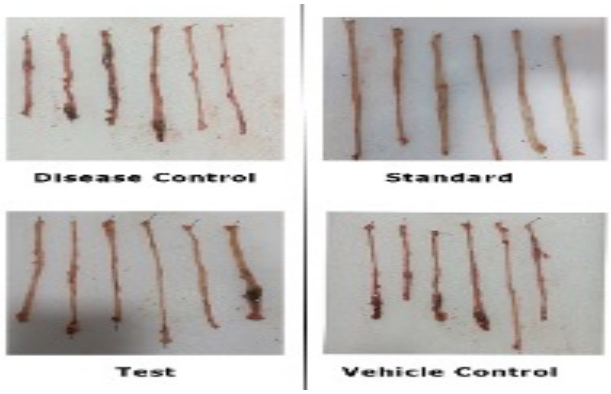
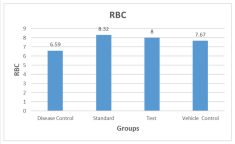


Figure 1: Macroscopic Assessment of Colon

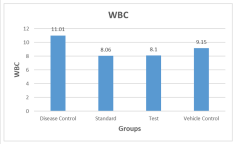


Haematological Parameter

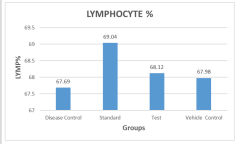
Graph 4: RBC (Red Blood Cells)



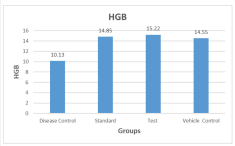
Graph 5: WBC (White Blood Cells)



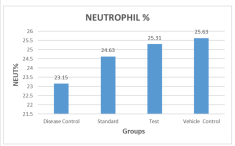
Graph 6 - Lymphocyte



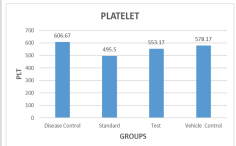
Graph 7: HGB (Haemoglobin)



Graph 8: Neutrophil



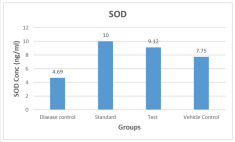
Graph 9: Platelet



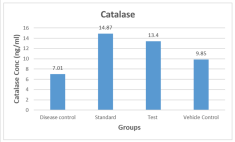
Assessment of Antioxidant Enzyme in the Colon

Highly significant changes were obtained in Standard group and Test group animals ($p<0.0001$) when compared to the disease control group. One-way ANOVA was used to find out difference between Disease Control group, Standard group and Test group.

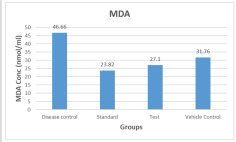
Graph: 10 Superoxide dismutase (SOD)



Graph 11: Catalase



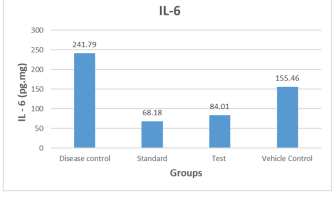
Graph: 12 Malondialdehyde (MDA)



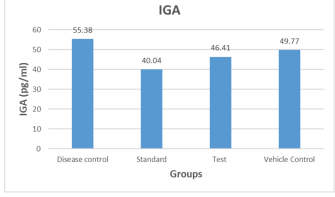
Serum IL-6 & Serum IGA parameter

Highly significant changes were obtained in Standard and Test group animals ($p<0.0001$) when compared to the disease control group. One-way ANOVA was used to find out difference between Disease Control group, Standard group and Test group.

Graph 13: Serum Interleukin -6 (IL-6)



Graph 14: Serum Immunoglobulin A (IGA)



Microscopic Assessment of Colon

Table 7: Histology Grading

Grade	Observation
0	Normal
1	Mild Toxicity
2	Moderate Toxicity
3	Severe Toxicity

Intrarectal administration of acetic acid evoked a colonic inflammation characterized by increased neutrophil infiltration, massive necrosis of mucosal and sub mucosal layers, sub mucosal ulceration, and increase in vascular dilation.

Figure 2: Disease Control Histopathology

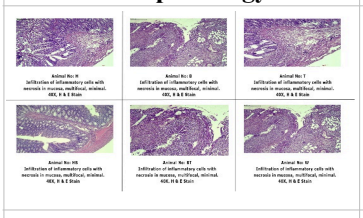


Figure 3: Standard Histopathology

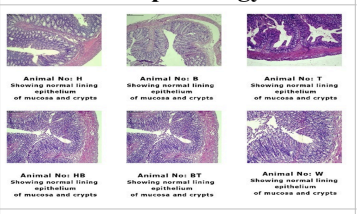


Figure 4: Test Histopathology

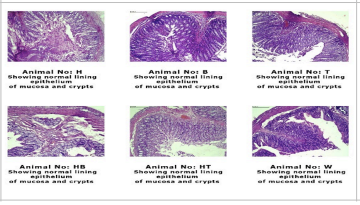


Figure 5: Vehicle Control Histopathology

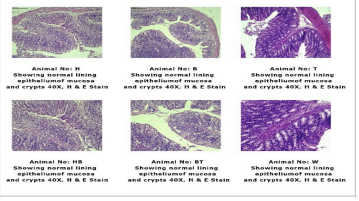
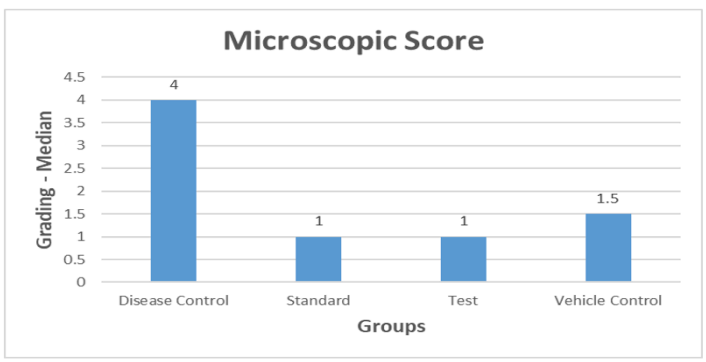


Table 8: Histological Scoring with Median

(Animal Marking on H= Head, B= Body, T= Tail, HB= Head and Body, BT= Body and Tail, W= No marking)

Animal marking	Disease Control	Standard	Test	Vehicle Control
H	4	1	1	1
B	2	2	0	2
T	4	1	3	1
HB	4	1	1	2
BT	4	0	2	1
W	4	2	1	3
Median	**4**	**1**	**1**	**1.5**
Interquartile range	**(3.5 – 4)**	**(0.75 – 2)**	**(0.75 – 2.25)**	**(1 – 2.25)**

Graph 15: Microscopic Score



A Kruskal Wallis test revealed a statistically significant difference in microscopic scores across all the four groups (p = 0.006)

Colon Weight-to-Length Ratio

Graph 16: Colon Weight/Length Ratio

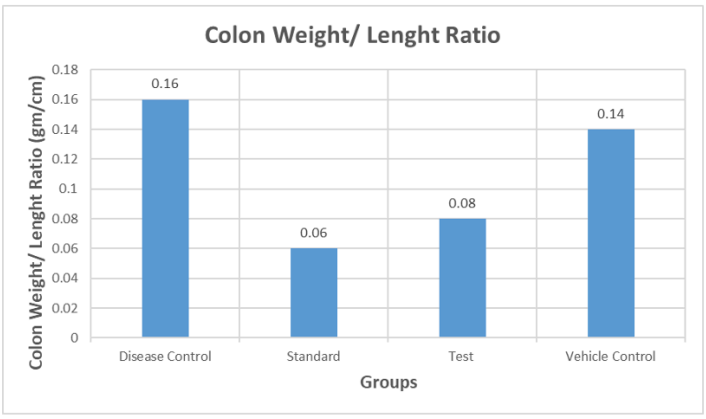


Table 9: Mean ± Standard Deviation (Colon Weight/ Length Ratio)

Groups	Day 8
Disease Control	0.16 ± 0.01
Standard	0.06 ± 0.00 (P Value ≤0.0001)
Test	0.08 ± 0.01 (P Value ≤0.0001)
Vehicle Control	0.14 ± 0.00 (P Value =0.0015)

Values are Mean ± **Standard Deviation**, n = 6 in each group.

Colon Weight-to-Length Ratio of Standard and Test group animal significantly decreased when compared to disease control group. In vehicle control, Colon Weight-to-Length Ratio was slightly decreased when compared to Disease control group. Disease control group increased Colon Weight-to-Length Ratio after Acetic acid treatment. P value of Standard and Test group was lower than 0.0001. Results were found to be significant. Double replicate ANOVA was used for the parameter **Colon Weight-to-Length Ratio** to find out difference between Disease control group and Test group.

Table 10: ANOVA: Two-Factor Without Replication

SUMMARY	Count	Sum	Average	Variance
Row 1	4	0.45	0.1125	0.002625
Row 2	4	0.43	0.1075	0.001492
Row 3	4	0.44	0.11	0.002267
Row 4	4	0.46	0.115	0.003033
Row 5	4	0.41	0.1025	0.001892
Row 6	4	0.44	0.11	0.002733
Column 1	6	0.96	0.16	0.0002
Column 2	6	0.37	0.061667	1.67E-05
Column 3	6	0.46	0.076667	2.67E-05
Column 4	6	0.84	0.14	0.00004

Table 11: ANOVA

Source of Variation	SS	df	MS	F	P-value	F crit
Rows	0.000371	5	7.42E-05	1.063745	0.418324	2.901295
Columns	0.041079	3	0.013693	196.3944	2.94E-12	3.287382
Error	0.001046	15	6.97E-05			
Total	0.042496	23				

Critical F value for significance 0.05 (DF1- 4, DF2- 6) is 3.287.
F Value obtained (196.3944) > Critical F value (3.287) at alpha=0.05

0.5 < Obtained P Value (0.0001) of Test drug < 0.001 (Good evidence against Null Hypothesis)

Therefore, Null Hypothesis is rejected and Alternate Hypothesis is accepted.

Hence, Alternate Hypothesis that is Rasanjanadi Churna will be effective in Acetic acid induced Ulcerative Colitis is accepted.

Discussion

Study was approved by the Animal Ethics Committee of the Institution. The present study was carried out on 24 Albino Wistar rats weighing 200-250gm after acclimatization for 7 days. Rats were randomly selected into 4 groups. Test Group was given *Rasanjanadi Churna* with *Tandulodak* and Honey, Vehicle Group was given *Tandulodak* and Honey, Standard Group was given Sulfasalazine and Disease Control Group was not given anything orally for 7 days. On day 4, colitis was induced in all groups by Intrarectal administration of 500 µL of acetic acid (4%). On 8th day, all the animals were sacrificed and following parameters were assessed.

Body weight: Intrarectal administration of acetic acid significantly reduced the body weight of rats in all groups. Loss of weight in colitis is due to deficiency of nutrients resulting from reduced appetite, food aversion or malabsorption, and rapid loss of body fluid through colorectal bleeding and diarrhoea. (Graph 1)

Clinical signs and symptoms: No mortality was observed during the study periods in Standard and Test group. No clinical signs such as lacrimation, salivation, irregular respiratory pattern, convulsion, tremors and no unusual behaviour were observed in the Standard and Test group. 3 animals were found dead in disease control group and 1 animal were found dead in vehicle control group during 8 days of observation period. All animals demonstrated comparable amount of feeds and water intake in rat during the duration of experiments.

Stool Assessment: Disease Control rats showed full diarrhoea with blood and Vehicle Control rats showed mild blood stool. In stool assessment, the grading Median for Disease control group was 3.5, Standard group was 1, Test Group was 2 and Vehicle control group was 2. (Table no. 3, 4)

Macroscopic assessment of the colon: Macroscopic examination of the colon revealed a significant increase in colon weight/length ratio of rats. This is due to severe tissue oedema, necrosis, goblet cell hyperplasia, and inflammatory cell infiltration. The macroscopic examination of intestinal content of Standard and Test group rats showed mild tissue oedema, necrosis, goblet cell hyperplasia, and inflammatory cell infiltration. Vehicle Control rats showed moderate tissue oedema, necrosis, goblet cell hyperplasia, and inflammatory cell infiltration. (Table no. 5, 6)

Haematological parameter: WBC and Platelets were increased in Disease Control group and, decreased in Test group. RBC and Haemoglobin were decreased in disease control animals and, increased in Test group. (Graph 4-9)

Biomarker Oxidative Assay: The acetic acid caused significant colon damage as indicated by a decrease in the level of Catalase (CAT) and Superoxide dismutase (SOD) and increase in the level of Malondialdehyde (MDA). Standard Drug and Test Drug significantly increased SOD & Catalase activity. Vehicle Control group showed slightly increased SOD activity and Catalase activity. Disease control rats showed increased the mean MDA level. Standard Drug and Test Drug significantly decreased the mean MDA level. Vehicle Control group slightly reduced MDA level. (Graph 10, 11, 12)

Serum IL-6 & IGA: Acetic acid significantly increased the mean Serum Interleukin -6 (IL-6) & Serum Immunoglobulin A (IGA) level. Standard Drug and Test Drug significantly decreased the mean Serum IL-6 & Serum IGA level. Vehicle Control group showed slightly reduced serum IL-6 & Serum IGA level. (Graph 13, 14)

Microscopic Assessment of Colon: Disease control rats showed high colonic inflammation with high infiltration and ulceration. Sulphasalazine rats and Test Group *Rasanjanadi Churna* showed mild colonic inflammation with less infiltration and ulceration. Vehicle control rats showed moderate colonic inflammation and ulceration. (Table no. 7, 8)

Colon Weight to length Ratio: The Disease control rats had increased mean colon weight/length ratio. Standard Drug and Test Drug significantly reduced the mean colon weight/length ratio. Vehicle Control group showed slightly reduced mean colon weight/length ratio. (Table no. 9)

Probable Mode of action of *Rasanjanadi Churna*

Treatment of Ulcerative Colitis is according to *Chikitsa* (treatment) protocol prescribed for *Raktatisaar* (bloody diarrhoea). Treatment protocol includes *Agni Dipana* (increase digestive fire), *Aama Pachana* (reduces indigestion), *Grahi* (absorptive action), *Stambhana* (anti-diarrheal), *Rakta stambhak* (Haemostatic), *Dhatu Poshaka* (nourishes body), *Sattvavajaya Chikitsa* (psychotherapy) according to the condition of the patient (12). All contents in *Rasanjanadi Churna* (Table no. 1) are *Grahi* (absorptive action), *Aama pachak* (reduces indigestion) and *Agni deepak* (increase digestive fire). *Rasanjan* (extract of *Berberis aristata DC*) (13), *Ativisha* (*Aconitum heterophyllum Wall. ex. Royle*) (14) (15), *Indrayav* (*Holarrhena antidysentrica(Wall)*) (16) (17) and *Kutaj* (*Holarrhena antidysentrica(Wall)*) (18) (19) have *Tikta Rasa* (bitter taste) and *Laghu* (light), *Ruksha Guna* (dry) so it pacifies *Pitta dosha* and reduces the *Drava Guna* (liquid) in body thereby controls *Atisaar* (diarrhoea). *Indrayav* (*Holarrhena antidysentrica(Wall)*) (16), *Kutaj* (*Holarrhena antidysentrica (Wall)*) (18) and *Dhataki* (*Woodfordia fructicosa (Linn.) Kurz.*) (20) (21) are *Shita* (cold) and have *Kashay Rasa* (astringent taste) so they control bleeding and *Atisaar* (Diarrhoea). *Shunthi* (*Zingiber officinale Rosc.*) (22) (23) is *Katu poushtik* (nourishes body), so it reduces the *Dhatu Daurbalya* (debility) which arises due to chronicity in Ulcerative Colitis. The vehicle for *Rasanjanadi Churna* are *Tandulodak* (24) (25) and honey (26), both are *Sthambhak* (anti-diarrheal) in nature which reduces *Atisaar* (Diarrhoea). Studies on ulcer healing property of honey (11), *Shunthi* (*Zingiber officinale Rosc.*) (27) and *Dhataki* (*Woodfordia fructicosa (Linn.) Kurz.*) (10) have already been carried out. Therefore, according to individual properties of drugs of *Rasanjanadi Churna* and properties of *Tandulodak* and Honey, the study drug may be beneficial in both *Aama* (immature) and *Pakwa* (mature) stages of *Raktatisaar* (bloody diarrhoea), i.e. (acute and chronic stages of Ulcerative colitis).

Conclusion

The findings of the Pre- Clinical study indicate that *Rasanjanadi Churna* showed positive results against the damaging effect of acetic acid induced colitis in Wistar rats. Activity of *Rasanjanadi Churna* was analysed for Macroscopic assessment and Microscopic assessment of colon, Haematological parameters, Stool assessment, Biomarker oxidative Assay, Serum

Interleukin-6, Serum Immunoglobulin-A parameters and Colon weight to length ratio respectively. *Rasanjanadi Churna* showed equivalent results in all the parameters when compared with Standard drug Sulphasalazine.

Rasanjanadi Churna has all herbal components so it can be consumed for longer duration. Ulcerative Colitis requires long term treatment so *Rasanjanadi Churna* is a better drug for its management.

Scope of the Study

Need to evaluate efficacy *Rasanjanadi Churna* clinically in patients of Ulcerative colitis.

Limitation of the study: There are no limitations for the study.

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