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STUDIES ON GUT HEALTH PROMOTING EFFECTS OF INTES'CARE (A POLYHERBAL FORMULATION) ON GI RELATED MEASUREMENTS IN EXPERIMENTAL ANIMALS

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ABSTRACT

Objective: The study is concerned with the evaluation of gut promoting effects of Intes'Care (a polyherbal formulation) on GI related measurements in experimental animals. **Methods:** Intes'Care is a polyherbal formulation developed by Suguna Foods Pvt Ltd. Acute oral toxicity was carried out in order to determine LD_{50}. In model I, acetic acid was used to induce the inflammatory bowel disease (IBD) in female Albino wistar rats on 1st and 3rd day. Treatment duration was for 15 days. On 15th day, physical parameters (body weight, food intake, water intake), blood parameters (RBC, WBC), LDH estimation, weight of the colon, colon mucosal damage index (CMDI) were recorded. Anti-diarrheal activity of Intes'Care powder (ICP) was evaluated using castor oil induced diarrhea and gastrointestinal motility testing in mice in Swiss albino mice. % diarrheal inhibition, total length of the intestine, peristaltic index were calculated. *In-vitro* nitric oxide scavenging activity of ICP was determined using Griess reagent. **Results:** Acute oral toxicity study showed no signs of toxicity by ICP even when the dose was increased to 2000mg/kg body weight. ICP improved the body weight, increased the feed as well as water intake. Significant increase in the RBC count and decrease in the WBC count when compared to IBD control rats was observed. Also increased LDH levels were brought back to near normal and decreased the CMDI scoring. ICP proved its antidiarrheal activity by reducing the onset of diarrhea, increasing the % diarrheal inhibition and peristaltic index. **Conclusion:** This study suggests that Intes'Care, a polyherbal preparation that is formulated by Suguna Foods Pvt Ltd, is effective in treating IBD, possesses antimotility, antidiarrheal effects in rodent model. Future studies investigating the role of Intes'Care in alleviating gastrointestinal disorders are certainly warrented.

KEYWORDS: Inflammatory bowel disease, diarrhea, Acetic acid, Castor oil, Sulfasalazine, Lopiramide.

INTRODUCTION

The gut is a fundamental organ system which makes up two equally important functions, i.e., the digestion and host defense.[1] Gut microbiota plays an important role in immunity and defences, digestion and metabolism, inflammation and cell proliferation. It is not only capable of communicating with the gut epithelium but also with different organs and bodily systems. Healthy gut microbiota is considered as the positive attribute, any changes and imbalances in gut microbiota are associated with altered health states.[2]

Nutrition-related disorders, inflammatory bowel disease (IBD) and certain allergies may be linked to varying compositions of the intestinal microbiome, and a better understanding of the gut microbiota will provide information essential for efficiently dealing with well-being and diseases such as obesity, the metabolic syndrome, food intolerance, IBD and irritable bowel syndrome (IBS).[3] To elicit the well-functioning and healthy gut, the dynamic balance of gut ecosystem is of importance. A wide range of factors related to diets and infectious disease agents seem to affect this balance, and subsequently affect the health status and production performance of the chicken. Stress may affect different physiologic functions of the gastrointestinal tract including gastric secretion, gut motility, mucosal permeability and barrier function, visceral sensitivity and mucosal blood flow.[4] Diarrhea is the 2nd leading causeof death across all ages next to lower respiratory infections.[5]

Certain medication used for illness also cause indigestion and other gastrointestinal distress Medications such as aspirin, Non-steroidal anti-inflammatory drugs (NSAID's), antibiotics, antidiabetic drugs (metformin),

antihypertensive medications (losartan), cholesterol lowering agents (clofibrate), antidepressant drugs, antiparkinsonian drugs, corticosteroids, estrgens, digoxin cause some form of GI distress.[6]

With the ban and/or reduction of the use of antibiotic growth promoters (AGPs) in poultry production, the alternatives to AGP are needed especially to preserve the balance of gut microbiota in chicken. Besides responsible for the absorption of nutrients from the lumen, intestinal mucosa of broiler chicken plays an important role in providing an effective barrier between the hostile luminal content and the host internal tissues. A subtherapeutic use of antibiotics has been widely practiced in poultry industry for decades to maintain the balance of ecosystem in the gut as well as to improve the growth performance of chicken. However, this practice has been questioned, given the increasing prevalence of resistance to antibiotics in chicken. Hence, alternatives to antibiotics are needed in poultry industry to maintain the gut health and promote the performance of birds.[7] The plant kingdom holds great potential to meet this need. Hence, in this study Intes'Care (a polyherbal formulation) is evaluated for its gut promoting effects of on GI related measurements in experimental animals.

MATERIALS AND METHODS

Chemical and drugs: Standard drugssuch as sulfasalazine, lopiramide were procured from medical store. Intes'Care, a polyherbal formulation was given from Suguna Foods Pvt Ltd. Castor oil was procured from the provision store.

Biochemical kits: The biochemical kits used for the estimation of LDH was procured from Anjan distributors, authorized supplier of ERBA Diagnostic Mannheim.

Experimental animals: Female Albino wistar rats, weighing 180-200g and Swiss albino mice, weighing 20-30g were used in this study. The animals were purchased from authenticated supplier Adita Biosys Private Limited, Tumakuru, Karnataka CPCSEA Registration No: 1868/PO/Bt/S/16/CPCSEA (with health certificate of the animals), and were maintained in the animal house of PES College of Pharmacy, Bengaluru. All the animals were acclimatized for 10 days under standard husbandry conditions, i.e. the animals were housed in polypropylene cages maintained under controlled temperature at $23^0C \pm 2^0C$, relative humidity 45-55% and with 12hrs light:12hrs dark cycle, temperature and humidity was recorded daily using thermometer and hydrometer mounted in animal house. The animals had free access to standard rat pellet along with water supplied *ad libitum* under strict hygienic conditions. Each experimental group had a separate set of animals and care was taken to ensure that animals used for one response were not employed elsewhere. Animals were habituated to laboratory conditions for 48 hours prior to experimental protocol to minimize if any of non-specific stress. The experimental protocols were approved by the Institutional Animal Ethics Committee (IAEC) approval No- PESCP/IAEC/56/2016, Dated: 14/11/2017) and conducted according to CPCSEA guidelines, Govt. of India.

Acute oral toxicity study[8]

Acute oral toxicity study was performed for newly developed polyherbal formulation according to the toxic class method 425 as per organization for economic cooperation and development (OECD) guidelines. The purpose of this study was to determine the LD50 of ICP. Female Albino wistar rats weighing between 180-200g were used for acute oral toxicity study. Prior to dosing, animals were fasted overnight. Using the default progression factor, doses were selected from the sequence 1.75, 5.5, 17.5, 55, 175, 550 and 2000 mg. As no estimate of Intes'Care's lethality is available, the dosing range chosen was between 175 mg/kg and 2000 mg/kg. The LD50 was calculated based on observations of physical and behavioral changes that were made for 14 days following the administration of the highest dose (2000 mg/kg body weight). The results indicated that mortality was not observed at 175, 550 and 2000 mg/kg body weight doses. Hence, 200mg/kg body weight (1/10th of 2000 mg/kg) was selected as the median dose for rat and $1/5^{th}$ dose that is 400mg/kg body wt p.o was selected as the high dose. From the rat dose, mice dose was calculated.

Acetic acid induced inflammatory bowel disease in rats[9,10]

Animals were divided into four groups of six rats in each group. Group I (normal) received water throughout the study till 14 days. Group II received acetic acid (1ml of 4%v/v, intra-colonic administration) which served as IBD control. Group III received acetic acid + standard (Sulfasalazine) 360mg/kg b.w.p.o. for 14 days. Group IV received acetic acid + polyherbal ICP (200mg/kg b.w.p.o) for 14 days. After overnight fasting of animals, under light chloroform anesthesia colitis (IBD) was induced by intra-colonic administration of 1 ml of 4% v/v acetic acid to all the groups except the Group I which was served as the normal control. (1^{st} day and 3^{rd} day of the 14 days study period). The food and water intake was monitored daily for each rat and the body weight of all the animals were recorded on the 1^{st}, 5^{th}, 10^{th}, 15^{th} day respectively using digital weighing balance.

Estimation of RBC and WBC count

On 15^{th} day, the blood was withdrawn from the individual animals of all the 4 groups by retro-orbital puncture under light ketamine anesthesia (40mg/kg i.p). One portion of blood was collected in an eppendorfs tube containing EDTA (anticoagulant) for the estimation of RBC and WBC and the other portion of the blood which was collected in the eppendorfs tube were kept in upright position for approximately 10-15min to facilitate clotting. The sample was centrifuged at 3000rpm for

15min. The separated serum was used for the estimation of LDH.

The rat blood was pipetted in the respective RBC & WBC pipette. Then the blood was diluted with RBC & WBC diluting fluid up to the mark and the contents of the pipette was mixed by rotating the pipette horizontally between the palms for 2-3min. The first two drops from the pipette were discarded and the Neubauers chamber was charged by putting a small drop of fluid to the central portion of slide from the pipette. The cells were allowed to settle for 2-5min. The chamber was placed on the stage of the microscope. The five squares for RBC and four squares for WBC were counted and the numbers of cells were noted down.[11,12]

Estimation of LDH

The enzyme lactate dehydrogenase (LDH) is concentrated in heart, kidney, liver, muscle and body tissues. Consequently, the damage to these organs results in increased serum levels of LDH. Elevated levels are associated with myocardial infarction, renal damage, hepatitis, anemia, malignancies and muscular disease or damage. The separated serum was used for the estimation of LDH and the procedure was followed according to the catalogue given in the ERBA kit. The concentration of all the samples was noted down directly using semi autoanalyser.

Evaluation of colon mucosal damage index

All the animals were sacrificed by injecting overdose of Pentobarbitone anesthesia intraperitoneally (150mg/kg b.w). The sacrificed rat was excised longitudinally and the colon segment was taken 10cm proximal to anus. The excised colon was rinsed with saline buffer solution. The colonic tissue was weighed using weighing balance and fixed in the wax block. Each colon was observed using magnifying glass or using electron microscope and evaluated. According to the method described by Morris *et al* and was given a score from 0-5. 0 = No damage, 1 = Localized hyperemia, but no ulcers or erosions, 2 = Ulcers or erosions with no significant inflammation, 3 = Ulcers or erosions with inflammation at one site, 4 = Two or more sites of ulceration and/or inflammation, 5 = Two or more major sites of inflammation and ulceration and ulceration or one major site of inflammation and ulceration extending >1cm along the length of the colon.[10]

***In-vitro* study- NO scavenging activity**

Sodium nitroprusside generates nitric oxide and was measured by Griess' reaction. Different concentration (0.2-0.8mg/ml) of ICP in ethanol was incubated with 2ml of 10mM sodium nitroprusside in 0.5ml of standard phosphate buffer saline (pH 7.5). Control solution did not contain ICP but it contained only the buffer solution in the identical manner. The above solution was incubated at room temperature for 5hrs. After 5hrs of incubation, 0.5ml of the incubation solution was removed from the solution and 0.5ml of Griess' reagent was added. This solution was incubated for 30 min at room temperature. The Chromophoric complex was formed as the result of diazotization of nitrite with sulphanilamide and its subsequent coupling reaction with nathylethylene diamine. The absorbance of the chromophore was measured at 546nm.[13]

$$\% \text{ Scavenging of NO} = \frac{\text{Abs of Control} - \text{Abs of Test}}{\text{Abs of Control}} \times 100$$

Castor oil induced diarrhea in mice

Twenty four Swiss albino mice were divided into four groups of six rats in each group. Group I animals received 0.5ml of castor oil orally (Disease control). Group II animals were treated with lopiramide at the dose of 3mg/kg orally and one hour after the administration of standard + 0.5ml of castor oil was administered respectively. Group III animals were treated with ICP (200mg/kg b.w.p.o) and one hour after the administration of test, 0.5ml of castor oil was administered respectively. Group IV animals were treated with ICP (400mg/kg.b.w.p.o) and one hour after the administration of test, 0.5ml of castor oil was administered respectively. The animals werekept in the metabolic cages and were observed for 4hrs. The onset of diarrhea and the weight of fecal output (wet and total feces in gram) was recorded for individual mouse. The percentages of diarrheal inhibition and weight of fecal output was determined according to the formula.[14,15]

$$\textbf{\% of inhibition} = \frac{\text{Average number of WFC} - \text{Average number of WFT}}{\text{Average number of WFC}} \times 100$$

Where, WFC = wet feces in the control; WFT = wet feces in the test group.

$$\textbf{Percentage of wet fecal output} = \frac{\text{Mean weight of wet feces of each group}}{\text{Mean weight of wet feces of control}} \times 100$$

$$\textbf{Percentage of total fecal output} = \frac{\text{Mean weight of total feces of each group}}{\text{Mean weight of total feces of control}} \times 100$$

Gastrointestinal motility testing in mice

Group I (Disease control) animals were administered with 0.5ml of castor oil orally. Group II (Standard) animals were treated with lopiramide (3mg/kg.b.w.p.o), half an hour after which 1ml of charcoal suspension administered and one hour after the administration of charcoal suspension, 0.5ml of castor oil was administered. Group III animals were treated with ICP (200mg/kg b.w.p.o), half an hour after which 1ml of charcoal suspension was administered and one hour after 0.5ml of castor oil was administered. Group IV animals were treated with ICP (400mg/kg b.w.p.o), half an hour after which 1ml of charcoal suspension was administered and one hour after the 0.5ml of castor oil was administered. The distance travelled by the charcoal meal, the total length of the intestine, the peristaltic index

and percentage of inhibition was calculated by using the following formula.[16,17]

Peristalsis index = Distance travelled by charcoal meal / Length of small intestine × 100

STATISTICAL ANALYSIS

All the values were expressed as mean ± SEM. Statistical comparisons were performed by one way analysis of variance (ANOVA) followed by Dunnett compare all column versus control column using Graph Pad Prism version 5.0. *P<0.05, **P<0.01, **P<0.001 was considered as significant compared to disease control.

RESULTS

Acetic acid induced inflammatory bowel disease in rats

Table 1: % change in body weight in normal and treated rats on 5th day.

Group	Group name	Body weight (g)		
		Initial wt	5th day	% change
I	Normal	211±8.21	201.1±13.3	4.6%- decrease
II	IBD	172.33±11.41	160±8.80	7.15%- decrease
III	IBD +Std	199.6±6.8	203.6±6.3	2.0%- increase
IV	IBD + ICP	196±6.09	163.6±6.15	16.5%- decrease

Table 2: % change in body weight in normal and treated rats on 10th day.

Group	Group name	Body weight (g)		
		5th day	10th day	% change
I	Normal	201.1±13.3	204.83±11.4	1.8%- increase
II	IBD	160±8.80	155.3±3.5	2.9%- decrease
III	IBD +Std	203.6±6.3	207.1±6.3	1.71%-increase
IV	IBD + ICP	163.6±6.15	168.6±6.1	3.05%- increase

Table 3: % change in body weight in normal and treated rats on 15th day.

Group	Group name	Body weight (g)		
		10th day	15th day	% change
I	Normal	204.83±11.4	205.83±10.1	0.48%- increase
II	IBD	155.3±3.5	147.83±3.51	4.81%- decrease
III	IBD +Std	207.1±6.3	212±6.30	2.3%- increase
IV	IBD + ICP	168.6±6.1	180.3±7.10	6.9%- increase

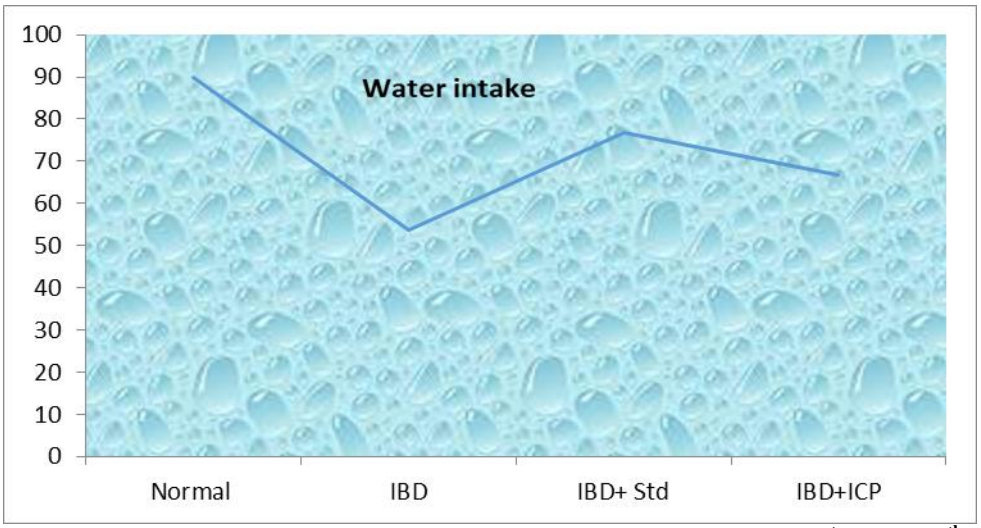

Fig. 1: Graph showing the feed intake of normal and treated rats from the 1st day to 15th day.

IBD control rats showed a decrease in their feed intake measured every day gradually throughout the study period (15 days) when compared to that of normal rats. Group III rats showed an improvement in the feed intakemeasured every day gradually throughout the study period (15 days) when compared to that of IBD rats. In group IV there was an improvement in feed intake measured every day gradually throughout the study

period (15 days) when compared to that of IBD rats. (Fig. 1).

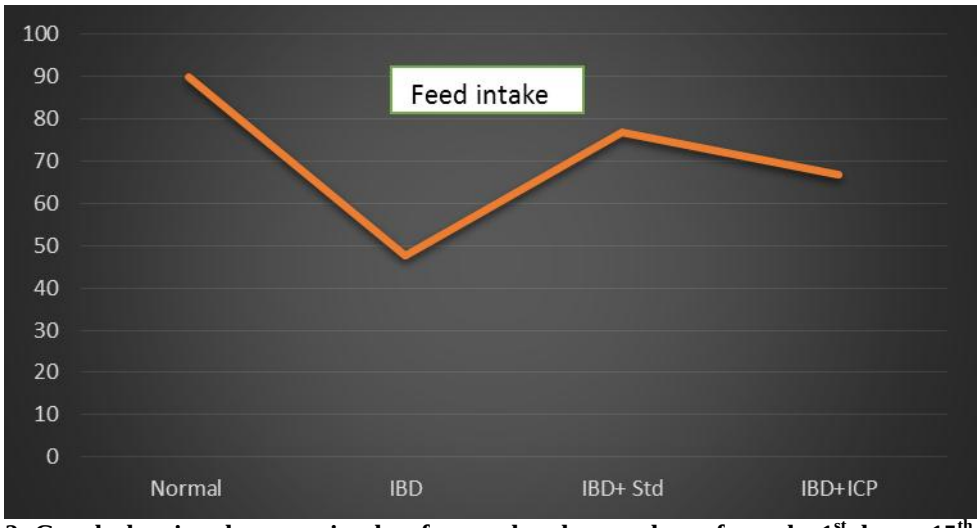

Fig. 2: Graph showing the water intake of normal and treated rats from the 1st day to 15th day.

IBD control rats showed a decrease in their water intake measured every day gradually throughout the study period (15 days) when compared to that of normal rats. Group III rats showed an improvement in the water intake measured every day gradually throughout the study period (15 days) when compared to that of IBD rats. In group IV there was an improvement in water intake measured every day gradually throughout the study period (15 days) when compared to that of IBD rat. (Fig. 2).

The results have shown a significant (P<0.0001) decrease in RBC count (4.9±0.31) in the IBD group when compared to the normal control group. The rats administered with standard Sulfasalazine (360mg/kg) and Intes'Care (200mg/kg) showed significant increase in RBC count (P<0.0001) when compared to the IBD control animals. Whereas, IBD control group rats have shown a significant (P<0.0001) increase in the WBC count (15.9±0.20) when compared to the normal control (6.31±0.19) animals. The treatment groups standard Sulfasalazine (8.39±0.67) and Intes'Care (200mg/kg) (9.56±0.37) have shown significant (P<0.0001) decrease in WBC count when compared to the IBD control group. (Table 4).

Table 4: Effects on hematological parameters in normal and treated rats.

Groups (n = 6)	Group name	Hematological parameters	
		RBC($\times10^6$µl)	WBC ($\times10^3$ µl)
I	Normal	8.5±0.53	6.31±0.19
II	IBD	4.9±0.31^{***a}	15.9±0.20^{***a}
III	IBD +Std	9.4±0.13^{***b}	8.39±0.67^{***b}
IV	IBD + ICP	7.6±0.52^{***b}	9.56±0.37^{***b}

The result have shown significant (P<0.0001) increase in serum LDH levels (2461.53±2.55) and weight of the colon tissue (2.91±0.14) in the IBD group when compared to the normal control group. The rats administered with standard Sulfasalazine (360mg/kg) at dose 200mg/kg showed significant decrease in serum LDH levels (980.85±2.47) and weight of the colon tissue (1.71±0.16) when compared to the IBD control animals. The rats administered with Intes'Care at dose 200mg/kg showed significant decrease in serum LDH levels (1457.21±2.06) and weight of the colon tissue (1.91±0.07) when compared to the IBD control animals. Whereas, IBD control group shows a significant (P<0.0001) increase in the colon mucosal damage index (4.66±0.23) scoring when compared to the normal control animals (0). The treatment groups standard Sulfasalazine (2.0±0.4) and Intes'Care shows significant (P<0.0001) decrease in colon mucosal damage index scoring (3.16±0.33) when compared to the IBD control group. (Table 5).

Table 5: Effects on serum LDH, weight of colon, CMDI score in normal and treated rats.

Group (n=6)	Group name	Serum LDH (U/I)	Weight of colon (g)	CMDI score
I	Normal	853.08±6.14	1.39±0.14	0
II	IBD	2461.53±2.55^{***a}	2.91±0.14^{***a}	4.66±0.23^{***a}
III	IBD +Std	980.85±2.47^{***b}	1.71±0.16^{***b}	2.0±0.4^{***b}
IV	IBD + ICP	1457.21±2.06^{***b}	1.91±0.07^{***b}	3.16±0.33^{***b}

Nitric oxide scavenging activity

In the present study as the concentration of Intes'Care has increased the radical scavenging activity was also elevated. When the concentration of the drug taken was 0.8µg/ml the radical scavenging activity was found to be 68.8% and as the concentration of the test substance increased to 1.2µg/ml the radical scavenging activity was also found to be 70.16%. Furthermore as the concentration of the ICP was increased, the percentage scavenging was also increased (directly proportional). (Table 6).

Table 6: Effect of Intes'Care on % Scavenging of nitric oxide.

S.No	Concentration of test substance (µg/ml)	Absorbance at 546nm	% Scavenging of NO
1	0.0 (Control)	0.305	-
2	0.8	0.095	68.8%
3	1.2	0.093	70.16%
4	1.6	0.091	70%
5	2.0	0.089	70.8%
6	2.4	0.087	71.4%
7	2.8	0.085	72.1%
8	3.2	0.081	73.44%

Castor oil induced diarrhea in mice

Group II rats treated with standard lopiramide (10mg/kg b.w) showed significant increase in the onset of diarrhea (132±4.33) when compared to the disease (62.5±3.39) control rats (diarrhea induced). An insignificant increase in the onset of diarrhea (81.66±5.03) was observed in group III which was treated with Intes'Care low dose (200mg/kg) when compared with group II. Group IV animals treated with Intes'Care high dose (400mg/kg) showed a highly significant increase in the onset of diarrhea (94.16±2.19). (Table 7).

Table 7: Effects on onset of diarrhea in normal and treated rats.

Group No	Group name	Onset of diarrhea (min)
I	Diarrhea control	62.5±3.39
II	Standard	132±4.33^{***a}
III	ICP (200mg/kg b.w)	81.66±5.03
IV	ICP (400mg/kg b.w)	94.16±2.19^{***b}

Group II rats treated with standard lopiramide (10mg/kg b.w) showed significant decrease in the average number of wet feces (1.08±0.20) when compared to the disease (3.78±0.48) control rats (diarrhea induced). A significant decrease in the average number of wet feces (2.15±0.12) was observed in group III which was treated with Intes'Care low dose (200mg/kg) when compared with group II. Group IV animals treated with Intes'Care high dose (400mg/kg) showed a significant decrease in the average number of wet feces (1.78±0.13) when compared with group II. Also, Group II rats treated with standard lopiramide (10mg/kg b.w) showed significant decrease in the average number of feces (1.45±0.21) when compared to the disease (4.33±0.53) control rats (diarrhea induced). A significant decrease in the average number of feces (2.83±0.19) was observed in group III which was treated with Intes'Care low dose (200mg/kg) when compared with group II. Group IV animals treated with Intes'Care high ssdose (400mg/kg) showed a significant increase in the average number of feces (2.11±1.42) when compared with group II. (Table 8).

Table 8: Effects on average No. of wet and total feces in normal and treated rats.

Group No	Group name	Avg No of wet feces (g)	Avg No of feces (g)
I	Diarrhea control	3.78±0.48	4.33±0.53
II	Standard	1.08±0.20^{***a}	1.45±0.21^{***a}
III	ICP 200mg/kg b.w)	2.15±0.12^{***b}	2.83±0.19^{***b}
IV	ICP 400mg/kg b.w)	1.78±0.13^{***b}	2.11±1.42^{***b}

Gastrointestinal motility testing in mice

Group II rats treated with standard lopiramide (10mg/kg b.w) showed significant decrease in the mean distance travelled by the charcoal meal (13.55±1.06) when compared to the disease (34.8±2.06) control rats (diarrhea induced). A significant decrease in the mean distance travelled by the charcoal meal (25.3±0.99) was observed in group III which was treated with Intes'Care low dose (200mg/kg) when compared with group II. Group IV animals treated with Intes'Care high dose (400mg/kg) showed a significant increase in themean distance travelled by the charcoal meal (12.68±1.26) when compared with group II. Also, Group II rats treated with standard lopiramide (10mg/kg b.w) showed significant decrease in the peristaltic index (31.33±2.47) when compared to the disease (81.48±2.49) control rats (diarrhea induced). A significant decrease in the peristaltic index (58.63±2.18) was observed in group III which was treated with Intes'Care low dose (200mg/kg) when compared with group II. Group IV animals treated with Intes'Care high sdose (400mg/kg) showed a significant decrease in the mean distance travelled by the charcoal meal (26.35±2.54) when compared with group II. (Table 9).

Table 9: Effects on mean distance travelled by charcoal and peristaltic index in normal and treated rats.

Group No	Group name	Mean distance travelled by charcoal (cm)	Peristaltic index
I	Diarrhea control	34.8±2.06	81.48±2.49
II	Standard	13.55±1.06^{***a}	31.33±2.47^{***a}
III	ICP 200mg/kg b.w)	25.3±0.99^{***b}	58.63±2.18^{***b}
IV	ICP 400mg/kg b.w)	12.68±1.26^{***b}	26.35±2.54^{***b}

DISCUSSION

In the present research work, acetic acid has been used as an agent for the induction of IBD in rats which is a best example for chemical induced colitis model because these models closely mimic morphological, histological and symtomatical features of human IBD A convenient approach to study the pathogenesis and complexity of human IBD is to induce IBD in animals. Animal models of IBD are indispensible for the proper understanding of histopathological and morphological changes in the intestinal tract. Consequently, animal models play pivotal role in the development of novel therapeutic drug to cure IBD and dissect the possible mechanism of action of a particular drug.[18]

General parameters – Model I- Acetic acid induced inflammatory bowel disease in rats

Body weight: The IBD rats showed a significant decrease in body weight throughout the study period (15 days) and this weight loss can be attributed due to a number of different processes. Since, IBD is inflammatory in nature, it results in generalized catabolic stage, exerts an anorexic effect by increasing resting energy expenditure, alterations in the levels of a number of hormones like leptin, adiponectin and ghrelin and also these disease process are associated with malbsorption of both macronutrients and micronutrients leading to the weight loss. IBD rats when treated with standard sulfasalazine (360mg/kg) showed a significant improvement in body weight throughout the study period and this is due to its anti-inflammatory activity. IBD rats when treated with ICP (200mg/kg) showed an insignificant increase in body weight on 5th day and 10th day and a mildly significant increase in body weight on 15th day when compared to IBD rats. Hence, this may be due to the reversal of all the events taking place during the process of inflammation.

Food intake and water intake: IBD control rats showed a decrease in the feed intake as well as water intake when compared to the normal rats and this may be attributed to food aversions since they associate eating with pain due to which eating is avoided in order to avoid pain or unpleasant symptoms. Also, depression is commonly seen in patients with IBD that can leads to the loss of appetite. When the IBD rats was treated with standard sulfasalazine and ICP it was found that there was a gradual improvement in the water as well as feed intake.[19]

Hematological parameters: RBC count: The IBD rats showed a significant decrease in RBC count and this is due two major reasons one is an impaired uptake, e.g., due to functionally disturbed absorption caused by inflammation of the ileum. The second major reason for an iron deficiency in IBD certainly is the continuous blood loss in active colitis or ileitis associated with a depletion of iron and iron stores. IBD rats when treated with standard sulfasalazine (360mg/kg) showed a highly significant increase in RBC count and this is due to its anti-inflammatory activity. IBD rats when treated with ICP (200mg/kg) showed a highly significant increase in RBC count when compared to IBD rats. Hence, this may be due to the reversal of all the events taking place during the process of inflammation.

WBC count: The IBD rats showed a significant increase in WBC count since IBD is associated with full-thickness inflammation (ie, the inflammation is transmural, involving all the tissue layers of the gastrointestinal lining). The inflammation associated with IBD is limited to the mucosal layer of colonic tissue. IBD rats when treated with standard sulfasalazine (360mg/kg) showed a highly significant decrease in WBC count. IBD rats when treated with ICP (200mg/kg) showed a highly significant increase in RBC count when

compared to IBD rats. Hence, this may be due to the reversal of all the events taking place during the process of inflammation.[21]

Serum parameter

Serum LDH: The IBD rats showed a significant increase in LDH levels. Lactate dehydrogenase activity in serum increases as a marker of cellular necrosis and contiguous tissue damaged liberates enzymes into circulation which contribute towards an abnormal increase in enzyme levels. IBD rats when treated with standard sulfasalazine (360mg/kg) showed a highly significant decrease in WBC count. IBD rats when treated with ICP (200mg/kg) showed a highly significant increase in RBC count when compared to IBD rats. Hence, this may be due to the reversal of all the events taking place during the process of inflammation.[22]

Weight of the colonic tissue and CDMI: The IBD rats showed a significant increase in weight of colonic tissue and the scoring of CDMI. This is due to the inflammation of the colonic tissue and also due to superficial damage which can be seen through the microscope. IBD rats when treated with standard sulfasalazine (360mg/kg) showed a highly significant decrease in colon weight and scoring of CDMI. IBD rats when treated with ICP (200mg/kg) showed a highly significant decrease in colon weight and scoring of CDMI when compared to IBD rats. Hence, this may be due to the reversal of all the events taking place during the process of inflammation.

Model II Castor oil induced diarrhea in mice

Onset of diarrhea: Group I rat administered with castor oil showed a highly significant decrease in the onset of diarrhea. Castor oil-induces diarrhea by releasing nitric oxide and thereby increasing permeability of gastrointestinal membrane for calcium, stimulating prostaglandin synthesis and thereby increasing fluid and electrolytes into the lumen of the bowel; and increasing peristalsis. Group II rats treated with standard lopiramide showed highly significant increase in the onset of when compared to the disease control rats (diarrhea induced) since loperamide exerts its antidiarrheal effect by a change in the motor function of the intestine, which results in increased capacitance of the gut and a delay in the passage of fluid through the intestine. An insignificant increase in the onset of diarrhea was observed in group III which was treated with Intes'Care low dose when compared with group II. Group IV animals treated with Intes'Care high dose showed a highly significant increase in the onset of diarrhea.

No of wet feces and total number of feces: Group I rat administered with castor oil showed a highly significant increase in the onset of diarrhea. Group II rats treated with standard lopiramide showed highlysignificant decrease in the average number of wet feces when compared to the disease control rats. A highly significant decrease in the average number of wet feces was observed in group III which was treated with Intes'Care low dose when compared with group II. Group IV animals treated with Intes'Care how dose showed a highly significant decrease in the average number of wet feces when compared with group II. Also, Group II rats treated with standard lopiramide showed highlysignificant decrease in the average number of feces when compared to the disease control rats (diarrhea induced). A highly significant decrease in the average number of feces was observed in group III which was treated with Intes'Care low dose when compared with group II. Group IV animals treated with Intes'Care low dose showed a highly significant increase in the average number of feces when compared with group II. The % inhibition of defecation was calculated and the results showed that the standard rats showed highly significant inhibition of defecation. ICP high dose showed better inhibitory action of defecation when compared to that of ICP low dose. Also the % wet fecal output and % total fecal output was calculated and the results showed that the standard rats showed highly significant inhibition in % wet fecal output and % total fecal output. ICP high dose showed better inhibitory action of % wet fecal output and % total fecal output when compared to that of ICP low dose.

Model III Gastrointestinal motility testing in mice

Group II rats treated with standard lopiramide showed significant decrease in the mean distance travelled by the charcoal meal when compared to the disease control rats. A highly significant decrease in the mean distance travelled by the charcoal meal was observed in group III which was treated with ICP low dose when compared with group II. Group IV animals treated with ICP low dose showed a highly significant increase in the mean distance travelled by the charcoal meal when compared with group II. Also, Group II rats treated with standard lopiramide showed significant decrease in the peristaltic index when compared to the disease control rats (diarrhea induced). A highly significant decrease in the peristaltic index was observed in group III which was treated with ICP high dose when compared with group II. Group IV animals treated with ICP high dose showed a significant decrease in the mean distance travelled by the charcoal meal when compared with group II.

CONCLUSION

The results of the study showed that Intes'Care (a polyherbal formulation) has a pronounced role in reducing the inflammation and mucosal damage in acetic acid induced inflammatory bowel disease which was at par with the standard drug used for the study i.e. Sulfasalazine. This concludes that the Intes'Care is an effective polyherbal formulation which can be used for the treatment of inflammatory bowel disease. Also the results revealed that Intes'Care (a polyherbal formulation) has a pronounced role in reducing the onset of diarrhea, number of wet fecal matter and total number of fecal matter. It was also effective in reducing the distance travelled by the charcoal meal as well as the

peristaltic index which indicated the anti-diarrheal potential of Intes'Care (a polyherbal formulation) was at par with the standard drug used for the study i.e. Lopiramide. Intes'Care (a polyherbal formulation) has proved to have a notable role in reducing the inflammation and mucosal damage by notably increasing the reduced body weight, water intake, feed intake and by reducing the levels of LDH, weight of the colon, colon mucosal damage index when compared to the disease control group i.e., Group II. It also was effective in scavenging the free radicals hence making it effective even as an efficient antioxidant activity. Since Intes'Care (a polyherbal formulation) was effective in treating inflammatory bowel disease as well as in diarrhea it may be concluded that our research work suggests that Intes'Care (a polyherbal formulation) developed by Suguna Life Herbs, Herbal division possess gut health promoting effect. The result data has provided useful discernment into the feasibility of using Intes'Care (a polyherbal formulation) as the gut health promoter. But further studies confirming its potential is certainly warrented.

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