Ensete superbum Roxb Cheesman (wild banana) peel extract ameliorates 2, 4, 6- trinitrobenzenesulfonic acid-induced colitis in rats

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Research Article

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Abstract

The wild banana plant *Ensete superbum* Roxb. Cheesman is widely used as traditional remedy among indigenous populations to relieve inflammation, stomachache and diarrhea. Earlier studies have revealed the *in-vitro* anti-cancer effects of peel dioxane fraction (PD) extracted from ripe peel aqueous extract (PA) of *E. superbum* on human colorectal adenocarcinoma cell lines. However, its gastro protective effect in complications of the inflammatory bowel disease (IBD) such as Ulcerative colitis (UC) has not been investigated. In the present work, the oral toxicity of PD was assessed, and its ameliorative effect in an animal model of ulcerative colitis was explored. In the acute and sub-acute toxicity trials, PD fraction's oral administration did not cause fatalities, and no notable changes relative to control were observed. The No-Observed Adverse Effect Level (NOAEL) of the PD fraction was assessed to be higher than 2000 mg/kg/day in rats. The ameliorative effect of varying concentration of PD fraction (100, 250 and 500 mg/k.g/b.w) in 2,4,6-trinitrobenzene sulfonic acid (TNBS) induced (intra-rectal administration) ulcerative colitis in rats was evaluated by comparing with the standard drug Sulfasalazine (100 mg/kg/b.w). Colon tissue ulceration with significant mucosal damage was observed in colitis rats, which waned in rats treated with PD fraction (500 mg/kg/b.w.), similar to that of rats treated with the sulfasalazine drug. The antioxidant, anti-proliferative, and anti-inflammatory capabilities of PD fraction may explain its protective impact; however, more study is needed to unravel the mechanisms of these pathways.

Introduction

Ulcerative colitis (UC) is an inflammatory ailment of the digestive tract, which is also known as inflammatory bowel disease (IBD). According to a meta-analysis, patients with UC have a high chance of contracting colorectal carcinoma, and the median cumulative risk of cancer occurrence has risen annually up to 18% after 30 years of UC. Clinical manifestations of UC are diarrhea, mucous pus discharge, blood in stool, abdominal discomfort, and tenesmus. The etiology of UC is complex and still not clear. Environmental variables, infections, immunological factors, and genetic predisposition may all have an influence in the development of UC [1, 2]. Currently, sulfasalazine (SASP) and mesalazine are the main drugs used for treating UC. Other treatments include the use of corticosteroids, antibiotics and immunosuppressive agents, and combination therapies. While combination drug therapies have some effectiveness, this condition recurs rapidly after the withdrawal of the drug. Unfortunately, prolonged usage of these treatments has serious complications and safety concerns, including drug addiction, adverse outcomes, a loss in immunological response, an elevated incidence of opportunistic infections, besides the development of lymphomas. Colectomy is required in 15% of ulcerative colitis patients in the long run [3, 4]. These limitations have triggered the need for novel and alternative holistic approaches to prevent UC.

Increasing evidence from several studies suggests that redox imbalance and colon inflammation play a part in the progression of UC. Therefore, these pathways could represent valuable therapeutic strategies for the development of novel medications [5, 6]. In this context, many therapeutic plant extracts have been evaluated in various models of UC. Some examples include apple polyphenols extract, green tea polyphenols, grape seed proanthocyanidin extract, pomegranate phenolic extract, aqueous extract of *Passiflora edulis* leaves [7, 8]. Experiments in *in- vitro* and *in-vivo* studies have shown that compounds from these medicinal plants are effective against IBD. Researchers have attributed these effects to the occurrence of one or more active chemicals, like polyphenols, alkaloids, flavonoids, coumarins, proanthocyanidins, terpenoids, and tannins [9, 10]. These compounds are reported to be potent anti-inflammatory and anti-cancer agents, especially in the colon, and some of them have also been investigated in human subjects [11]. Health beneficial/ nutrient rich foods high in phytochemicals and antioxidants are useful because of their natural capacity to scavenge free radicals, stimulate anti-inflammatory responses, maintain normal balance of the intestinal flora, and trigger the intestinal T regulatory cells [12, 13]. These studies highlight the need to determine the potential

efficacy of new therapeutics in modifying or modulating molecular systems including oxidative and inflammatory parameters to prevent or ameliorate UC.

The family Musaceae includes banana which is the most commercially significant plant in the order Zingiberales. Ensete and Musa, two of the three genera in the family Musaceae representing seeded wild type to seedless cultivars are found in India. Among them, the seeded wild banana *Ensete superbum* Roxb. Cheesman can be found in the Western Ghats, Aravalli hills and Northeastern hills of India. Some of the findings on *E. superbum* as a traditional medicinal source highlight the use of crushed seeds in water for stomachache, seed paste for fever with body pain; pseudostem juice for diarrhea, dysentery and food poisoning; whole fruit for stomachache, fever and typhoid; and powdered whole plant for stomachache, fever, cold and cough [14]. Previously we have demonstrated that the aqueous extracts (ripe peel, seed, flower and bract) of *E. superbum* are rich in health-promoting phytochemicals and possess high antioxidant potential [15]. Furthermore, the ripe peel aqueous extract (PA), its fraction (PD) and isolated compounds possessed cytotoxic activity [16]. The present work, evaluated the *in-vivo* toxicity of dioxane fraction from *E. superbum* peel aqueous extract and established its therapeutic potential in a rat model of UC.

Materials And Methodology

The materials and methodology section is presented as Supplementary file

Results And Discussion

Oral toxicity assessment of PD fraction

The oral administration of PD fraction at all dose levels did not cause any fatalities or clinical indications of toxicity in rats, in both acute and subacute toxicity trials. No significant body weight variations were observed between the control and the PD test groups and the groups exhibited a typical incremental gain in body weight (Fig.1 a, c). On gross examination of vital organs, no histological alterations or damages were observed, and the relative organ weight was found to be statistically similar ($p>0.05$) between the control and PD test group (Fig.1 b, d). The hematological and serum biochemical profile of both the toxicity studies is summarized in Table 1, and the results indicated that all hematological parameters are within the normal range in both control and PD test groups. No significant difference was evidenced in liver and kidney function parameter values relative to the control group.

Anti-colitis effects of PD fraction in Trinitrobenzene sulfonic acid induced animal Ulcerative colitis model

Disease activity index (DAI) score

There were no fatalities in the experimental groups. A single-dose of TNBS enema in the colitis model population caused acute colitis, diarrhea and hemoccult, followed by severe weight loss from day 5 to day 20 as observed by the DAI score (Fig. 2a). In the Sulfasalazine treated groups, bloody stools were not observed and stools were intact like pellets, and rats showed less weight loss. DAI score was decreased in the rats fed with PD fraction in a dose- dependent manner as compared to TNBS model rats. Previous studies on oral toxicity of *E. superbum* extracts and fractions isolated from seeds and pseudostem were found to be non-toxic (LD50 = 3235.9 mg/kg) [14]. In our study the No-Observed Adverse Effect Level (NOAEL) of the PD fraction was valued to be higher than 2000 mg/kg/day in rats. Hence, it can be concluded that PD fraction is safe for oral administration.

Colon tissue assessment

Microscopic examination of the healthy control population's colon tissue showed that it was complete, intact and free of damage with a histological colitis score of zero (Fig 2b, c). In the UC model group, there was a severe disruption of colon tissues with ulceration and inflammation involving the epithelium layer and all intestinal layers. The inflammatory response was consistent with the transmural invasion of inflammatory cells resulting in structural distortion of crypts, epithelial degradation and goblet cell depletion. The UC model had the highest histological scoring for colitis ($p<0.001$) with corresponding high score for inflammation and crypt damage (Fig. S1 a-d & Fig. 2b, c). However, rats treated with Sulfasalazine and PD fractions showed a gradual healing of colon tissue impairment with a lower colitis score and fewer ulceration and severities (Fig S1 a-d & Fig. 2b, c). This was also evidenced by a lower tissue regeneration score (Fig. S1 d & Fig. 2c). The efficacy of PD on TNBS-induced colonic injury was dose-dependent, with 500 mg/kg being significantly more efficacious than 100 mg/kg (Fig. S1 c-d & Fig. 2c).

Colon tissue redox/oxidative imbalance markers

The function of redox/oxidative imbalance in laboratory animals and human participants with UC has been widely researched, with higher ROS levels coupled by decreasing antioxidant activity in the inflamed mucosa, resulting in greater chronic tissue injury [17]. Thus, the UC mucosa may be in a continual state of redox imbalance, posing a major danger to intestinal tissue homeostasis and increasing endogenous antioxidant demand. Therefore, these mechanisms may provide useful therapeutic strategies for the development of novel medications [18, 19].

Furthermore, suppressing ROS-inducing enzymes, active quenching of ROS, or improving cellular antioxidant reserves are some of the interventions for UC treatment [20]. The dominant cellular antioxidants expressed or released into the blood stream following a disease-related event are superoxide dismutase (SOD), glutathione (GSH), and catalase (CAT). They are often used as biomarkers for inflammation and oxidative stress [21, 22]. Numerous experiments have shown that SOD levels decrease under UC environments. In contrast to these results, studies have also indicated increased SOD activity. The above inconsistent outcomes may be attributed to an active SOD protection mechanism in the disease situation leading to a rise in the activity, and the reduction in activity could be attributed to the utilization of the SOD enzyme to compensate for tissue damage and inflammation [23, 24]. The antioxidant markers analyzed in colon samples are presented as Table 2. In our experiment, Superoxide dismutase (SOD) activity was decreased ($p<0.001$) in colon tissue samples of the UC model population (4.177 U/mg protein). Whereas, the SOD antioxidant function improved in rats treated with Sulfasalazine (10.5) and PD500 (11.4), with function statistically comparable ($p>0.05$) to the healthy control group (12.6) (Table 2). Catalase a hydrogen peroxide-degrading enzyme is found in the cytoplasm and peroxisomes of the colon epithelial and lamina propria, and is triggered when hydrogen peroxide levels rise during an inflammatory response [25]. In this study, tissue catalase antioxidant activity is decreased in the UC model population (13.3 U/mg protein), and the difference was statistically significant ($p<0.001$) as compared to the activity in the normal control rats (50.5). The catalase function in rats treated with sulfasalazine (25.8) and all doses of PD fraction (25.4, 26.3, 26.6) were statistically comparable to each other, with an increase in enzyme activity when compared to the UC model population.

Glutathione is a tripeptide composed of cysteine, glycine, and glutamic acid and occurs as reduced (GSH) and oxidized (GSSG). Glutathione deficit in the intestinal mucosa has been linked to UC, and it is routinely assessed as a sign of *in-vivo* lipid peroxidation and redox/oxidative imbalance [20]. Glutathione-S-transferases, glutathione peroxidases (GPx), and glutathione reductase (GR) are antioxidant enzymes active in the GSH cycle that shield the intestinal mucosa from redox/oxidative and inflammatory injury [26]. The tissue glutathione reduced, peroxidase and reductase function was shown to be depleted in the UC model population (0.7 nmol/GSH/min/mg protein, 0.11 U/mg protein and 6.9 U/mg protein, respectively), and was statistically significant ($p<0.001$) to the healthy rat population (2.0, 0.46 and 20.0, respectively). In contrast, the reduced glutathione, glutathione peroxidase, and reductase enzyme functions were increased in the PD500 group (1.3, 0.33 and 13.3, respectively), with statistically ($p>0.05$) similar results to the

Sulfasalazine population (1.4, 0.36 U/mg and 13.6, respectively). Furthermore, membrane lipids in UC are vulnerable to ROS-induced disruption, resulting in lipid peroxidation products reflected in malondialdehyde (MDA) levels [27]. The PD500 treatment caused a marked reduction in tissue MDA levels (0.04μmol MDA/mg protein), indicating that the PD fraction effectively reduced TNBS induced lipid peroxidation, which was greater ($p<0.001$) in the UC group (0.08). These data demonstrate that PD500's activity and underlying mechanism of protection against UC may be due to its ability to influence redox hemostasis, reducing the severity of tissue damage and inflammation. Additionally, biological samples contain a plethora of antioxidant substances both in the aqueous phase (urate, ascorbate, bilirubin, and thiols) as well as in lipophilic phase (α-tocopherol, carotenoids, and flavonoids) [21, 28]. Previous investigations have highlighted the presence of polyphenols such as quercetin-3-O-rutinoside, 3,5-dimethoxy-4-hydroxybenzoic acid, and 4',5,7-trihydroxyflavone in the PD fraction [16]. The beneficial properties of polyphenols found in the fraction, as well as their synergistic interactions with the antioxidants present in the tissue, might explain the PD fraction's enhanced antioxidant capacity and efficacy in UC.

Colon tissue inflammation markers

The heme protein myeloperoxidase (MPO), which is naturally generated by phagocytes, particularly neutrophils, is a diagnostic for localized leukocyte aggregation and inflammation. MPO activation has been related to disease severity in UC patients with inflamed colon biopsy [29, 30]. The current study found a substantial increase in MPO activity (1.13 mU/g tissue), indicating an alleviation of colon inflammation in the UC model population. On the other hand, the control population showed residual MPO activity (0.25), which is consistent with the absence of an inflammatory process. The rats treated with PD fraction improved their MPO function in a dose-dependent way, and the effects of PD500 (0.36) were similar to those of Sulfasalazine (0.34) (Table 2). Overall, the drop in colonic MPO function and the histologic observations of reduced cellular infiltration (Fig. 2b, c) following PD500 administration show that it has potent anti-inflammatory effects.

Conclusion

As the usage of plant-based products grows, it is critical to examine the toxicological profile of these botanicals to validate their safety and effectiveness. In our study, PD's oral administration did not show any mortality, and the No-Observed Adverse Effect Level (NOAEL) was estimated to be more than 2000 mg/kg/day in rats indicating that it is safe for oral administration. The administration of TNBS into the rat colon produced an acute colitis model, which is an effective UC laboratory model for investigating acute and chronic colon mucosa injury, as well as the biochemical processes related with free radical generation and inflammatory response. The UC model was distinguished by an enhanced DAI score and also by macro and microscopic damage to the colon tissue. Varying amounts of tissue antioxidant enzymes was observed in colitis rats. The therapeutic effectiveness of the PD fraction was observed at a dose of 500 mg/kg. This dosage decreased macroscopic and microscopic intestinal damage, as assessed by the area of damaged tissue, and relieved the histological lesions observed in the animal colitis model. The beneficial effect of PD fraction at 500 mg/kg dose was due to the acceleration of the healing process, and it was seen during the 2 weeks after colonic insult. This is the first research to demonstrate that *E. superbum* fraction has *in-vivo* gastroprotective properties in experimental colitis. Our findings suggest that taking PD orally has no toxicity and that it may have favorable benefits in UC by reducing redox/oxidative imbalance and inflammatory reactions. Further approaches are needed to determine which molecular pathways are involved in these effects.

Abbreviations

CAT
Catalase

CPCSEA
Committee for the Purpose of Control and Supervision of Experiments on Animals
DTNB
5, 5-dithiobis-2-nitrobenzoic acid
EDTA
Ethylenediaminetetraacetic acid
GR
Glutathione reductase
GSH
Glutathione
IAEC
Institutional Animal Ethical Committee
IBD
Inflammatory bowel disease
MDA
Malondialdehyde
MPO
Myeloperoxidase
NOAEL
No-Observed Adverse Effect Level
SASP
Sulfasalazine
SOD
Superoxide dismutase
TNBS
2,4,6-Trinitrobenzenesulfonic acid
UC
Ulcerative colitis

Declarations

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Declaration of interests

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Author Contribution

Nimisha Sarah Mathew: Formal analysis, Writing- Original draft preparation. S. P. Muthukumar: Methodology, Data curation, Writing- Reviewing and Editing. B. K. Bettadiah: Data curation, Methodology, Writing- Reviewing and Editing. P. S. Negi: Conceptualization, Methodology, Data curation, Writing- Reviewing and Editing.

Availability of data and material

Not applicable

Ethics approval

Institutional Animal Ethical Committee (IAEC No. FT/AHF/ 26th IAEC/12/14) clearance was obtained for conducting *in-vivo* experiments, following the guidelines of the Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA), Ministry of Fisheries, Animal Husbandry and Dairying, Govt. of India, New Delhi.

Consent to participate

Not applicable

Consent for publication

All authors have given consent to publish.

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Tables

Table 1. Hematological and serum biochemical markers of oral toxicity studies

Hematological analysis

Parameter	Acute toxicity		Sub-acute toxicity	
	Cont	PD^{I}	CONT	PD^{II}
Red Blood Cells x10^6/ml	7.5±0.07^a	7.7±0.2^a	7.6±0.2^a	7.3±0.3^a
Hemoglobin g/dl	14.5±0.10 ^{a}	14.1±0.2 ^{a}	13.9±0.2 ^{a}	13.7±0.4 ^{a}
Hemocrit %	43.8±0.2 ^{a}	42.8±0.9 ^{a}	41.8±0.9 ^{a}	41.9±1.1 ^{a}
Mean Corpuscular Volume fl	58.7±0.7 ^{a}	53.5±4.3 ^{a}	53.5±0.8 ^{a}	57.0±0.9 ^{a}
Mean Corpuscles Hemoglobin pg	19.2±0.05 ^{a}	18.2±0.6 ^{a}	16.7±3.0 ^{a}	18.7±0.2 ^{a}
Mean Corpuscles Hemoglobin concentration g/dl	33.1±0.1 ^{a}	33.1±0.3 ^{a}	33.1±0.9 ^{a}	32.9±0.3 ^{a}
Platelets x10^3/ml	618±55 ^{a}	670±89 ^{a}	541±26 ^{a}	546±23 ^{a}
Parameter	**Serum biochemical markers (Liver and kidney function)**			
	Cont	PD^{I}	Cont	PD^{II}
Albumin (g/dl)	3.4±0.1 ^{a}	3.4±0.07 ^{a}	2.9±0.3 ^{a}	3.0±0.2 ^{a}
Total protein (g/dl)	6.3±0.3 ^{a}	6.4±0.1 ^{a}	4.8±0.3 ^{a}	4.9±0.1 ^{a}
Bilirubin total (mg/dl)	0.2±0.06 ^{a}	0.2±0.03 ^{a}	0.1±0.01 ^{a}	0.1±0.02 ^{a}
Bilirubin direct (mg/dl)	0.08±0.02 ^{a}	0.09±0.02 ^{a}	0.05±0.01 ^{a}	0.07±0.008 ^{a}
Creatinine (mg/dl)	0.2±0.1 ^{a}	0.2±0.03 ^{a}	0.11±0.008 ^{a}	0.11±0.001 ^{a}
Uric acid (mg/dl)	4.5±0.3 ^{a}	4.3±0.1 ^{a}	2.9±0.6 ^{a}	2.9±0.8 ^{a}
Urea (mg/dl)	24.2±4.4 ^{a}	27±1.4 ^{a}	16.4±2.2 ^{a}	14.6±3.6 ^{a}
Blood urea nitrogen (mg/dl)	13.2±5.2 ^{a}	13.5±3.3 ^{a}	7.1±1.7 ^{a}	7.3±1.5 ^{a}
Alkaline phosphatase (U/L)	117±13.5 ^{a}	113±15.3 ^{a}	27.3±3.0 ^{a}	24.9±4.7 ^{a}
Creatine kinase (U/L)	68.2±8.0 ^{a}	67±9.8 ^{a}	43.9±9.2 ^{a}	45±9.9 ^{a}
Gamma glutamyl transferase (U/L)	2±0.8 ^{a}	1.9±0.6 ^{a}	1.6±0.6 ^{a}	1.7±0.4 ^{a}
Alanine aminotransferase (U/L)	48.8±9.4 ^{a}	32.5±6.4 ^{a}	29.9±3.2 ^{a}	20.7±3.4 ^{a}
Aspartate aminotransferase (U/L)	67.9±4.3 ^{a}	65.6±4.5 ^{a}	59.6±4.0 ^{a}	57.3±5.3 ^{a}

Values are presented in row under each study group as mean ± S.D (n = 3: acute toxicity, n=5: sub-acute toxicity) and values followed by same letter are statistically same (p> 0.05)

Cont- Normal rats received 1% DMSO prepared in RO water

PD[!]- Rats received peel dioxane fraction dissolved in 1% DMSO with increasing graded dosages from 100, 200, 250, 500, 750, 1000 and 2000mg/kg/b.w. on alternate days

PD[!!]- Rats received peel dioxane fraction dissolved in 1% DMSO at 500mg/kg/bw of PD every day for 28 days

Table 2. Effects of varying concentrations of PD fraction on tissue antioxidant and anti-inflammatory parameters in Trinitrobenzene sulfonic acid induced animal Ulcerative colitis model

Tissue antioxidant parameters in UC						
Antioxidant enzymes	**CONT**	**TNBS**	**T+Sulfa**	**T+PD100**	**T+PD250**	**T+PD500**
Superoxide dismutase (U/mg protein)	12.6 ± 0.4^{a}	4.17 ± 0.1^{b}	10.5 ± 0.3^{c}	6.1 ± 0.2^{d}	7.3 ± 0.4^{e}	11.4 ± 0.5^{ca}
Catalase (U/mg protein)	50.5 ± 2.2^{a}	13.3 ± 2.2^{b}	25.8 ± 1.4^{c}	25.8 ± 0.7^{c}	26.2 ± 0.6^{c}	26.6 ± 1.1^{c}
Glutathione reduced (nmol/GSH/min/mg protein)	2.0 ± 0.06^{a}	0.7 ± 0.01^{b}	1.4 ± 0.05^{c}	1.1 ± 0.009^{d}	1.2 ± 0.5^{de}	1.3 ± 0.02^{ce}
Glutathione peroxidase (U/mg protein)	0.4 ± 0.01^{a}	0.11 ± 0.004^{b}	0.36 ± 0.025^{c}	0.23 ± 0.009^{d}	0.31 ± 0.001^{e}	0.33 ± 0.01^{ce}
Glutathione reductase (U/mg protein)	20.0 ± 0.6^{a}	6.9 ± 0.1^{b}	13.9 ± 0.5^{c}	11.0 ± 0.08^{d}	11.9 ± 0.5^{de}	13.3 ± 0.2^{ce}
Lipid peroxidation	0.03 ± 0.001^{a}	0.08 ± 0.002^{b}	0.04 ± 0.001^{c}	0.06 ± 0.003^{d}	0.05 ± 0.001^{e}	0.04 ± 0.0009^{ce}
Tissue anti-inflammatory parameter in UC						
Inflammatory enzyme	**CONT**	**TNBS**	**T+Sulfa**	**T+PD100**	**T+PD250**	**T+PD500**
Myeloperoxidase (mU/g tissue)	0.25 ± 0.01^{a}	1.13 ± 0.03^{b}	0.34 ± 0.01^{c}	0.63 ± 0.03^{d}	0.43 ± 0.02^{e}	0.36 ± 0.01^{ce}

Values are presented in row under each study group as mean ± S.D (n =6) and values followed by same letter are statistically same ($p> 0.05$)

Cont- Normal rats received 1% DMSO in RO water

TNBS-TNBS induced ulcerative colitis rats received 1% DMSO in RO water

T+Sulfa- TNBS induced ulcerative colitis rats administered Sulfasalazine (100 mg/kg b.w.)

T+PD100, T+PD250 and T+PD500- TNBS induced ulcerative colitis rats received peel dioxane fraction dissolved in 1% DMSO at 100, 250 and 500 mg/kg b.w, respectively

Figures

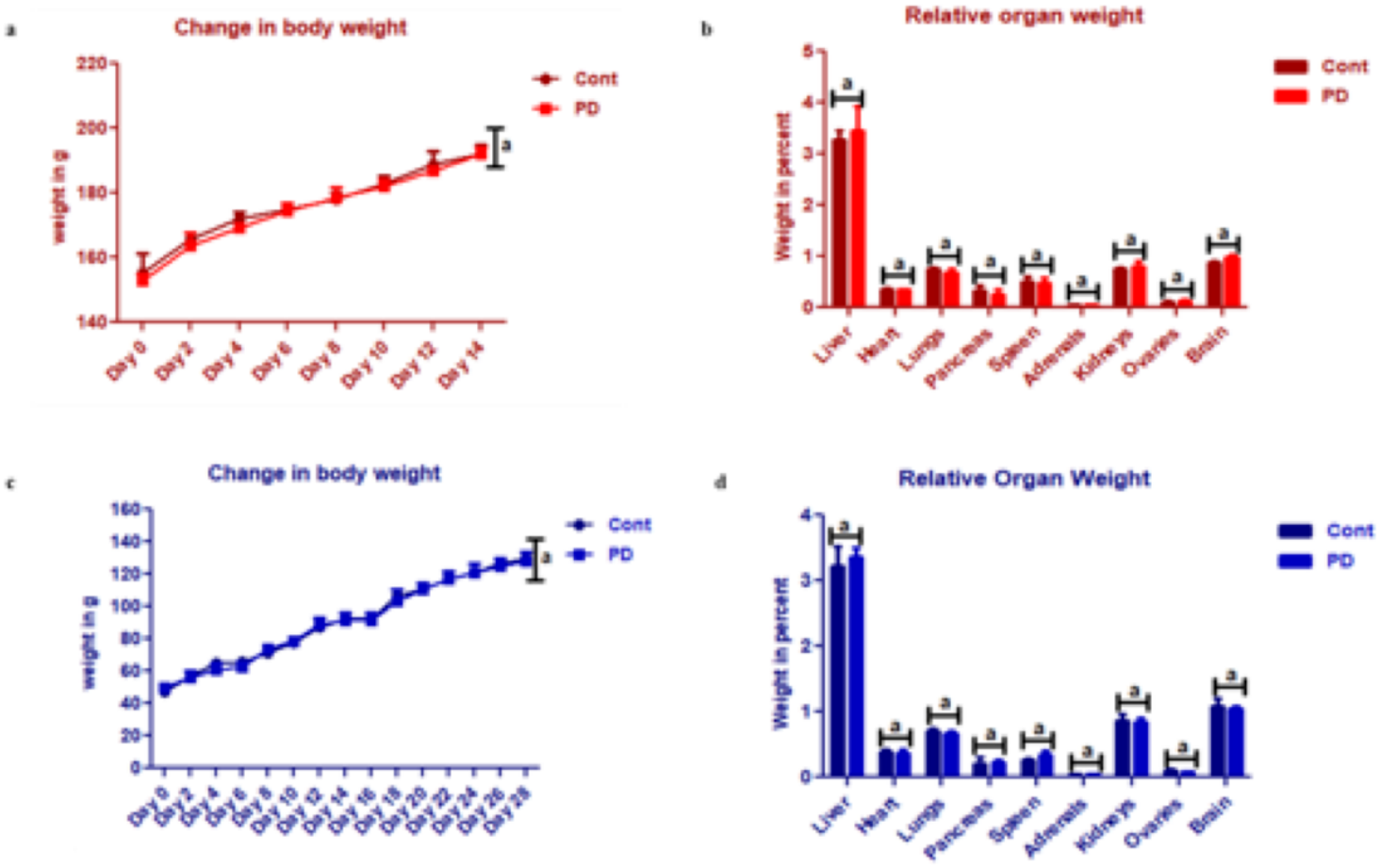

Figure 1

Body weight and relative organ weight observed in oral toxicity studies of PD fraction

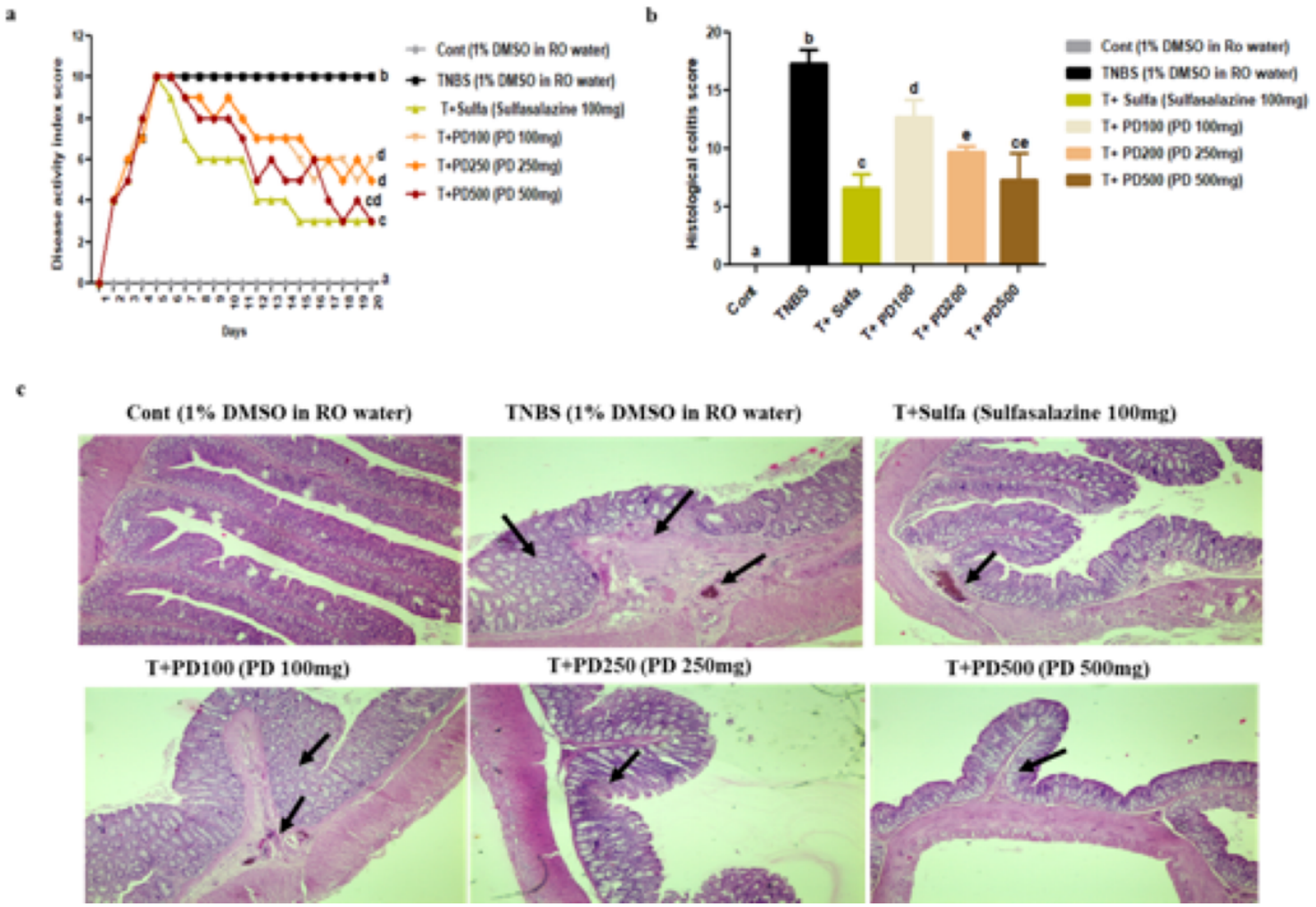

Figure 2

Anti-colitis effects of varying fractions of PD in Trinitrobenzene sulfonic acid induced animal Ulcerative colitis model

Supplementary Files

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- Supplementary.docx