

Anti-inflammatory Effect of *Basella rubra* on Oxazolone-induced Colitis in Rat

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ABSTRACT

A pilot study was conducted to study the phytochemical and anti-inflammatory activities of methanol extract of leaves of *Basella rubra*. Phytochemical studies were carried out to determine the bio active constituents present in the methanol extract of *B. rubra*. Oxazolone induced inflammation model was used to evaluate the methanolic extract of *B. rubra* for its anti-inflammatory activity. Treatment of animals with methanol extract of leaves of *B. rubra* at dose level of 500mg/kg b.w. was performed and found to possess marked activity of cure of inflammation induced by Oxazolone. The changes in body weight, colon structure, histopathological changes and inflammation score were examined between control and treated groups. Rats treated with only Oxazolone showed changes in hematoxylin and eosin sections with wide areas of mucosal necrosis with elongated irregularly branched glands including loss of differentiation (goblet cells). Treatment with methanol extract of leaves of *B. rubra* significantly recovered in the colon inflammation. The results of our study suggest that methanolic extract of leaves of *B. rubra* has beneficial effects on the experimental induced inflammation. This reveals that *Basella rubra* could be serves as a potential anti -inflammatory and chemotherapeutic drug.

Keywords: *Basella rubra*, Oxazolone, Inflammation score, Phytochemicals.

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INTRODUCTION

Inflammation in the gastrointestinal tract is referred as inflammatory bowel disease (IBD). Two types of IBD are Chron's disease and Ulcerative colitis (UC) these share the general clinical symptoms.^{1,2} IBD is associated with the activation of inflammatory mediators, signs of oxidative

stress, a deranged colonic milieu, increased intestinal permeability.³ It is believed that current drugs used for treatment of IBD having side effects. So far now there is no drug without any secondary effects.⁴ New therapies are in development to target the disrupted process. For that reason, the

current research is focused on screening the economical, easily accessible drug from natural source. Different plants have been used as a source in the progress of novel drugs either in pure compound or their extract form. Plant original medicines are extensively used because they are relatively safer than the synthetic drugs.⁵ The medicinal property of plant lies in secondary metabolites that produce a distinct physiological action and enormous benefits to human health.⁶ The most significant of these bioactive compounds of plants are alkaloids, flavonoids, tannins and phenolics.⁷ Therefore we conducted phytochemical tests to assess chemical nature of extracts qualitatively. The plant under investigation *Basella rubra*, known to be Malabar spinach or cyclone spinach, belongs to the family Basellaceae. It is widely used as green vegetable throughout the country and in many other parts of the world.⁸ *Basella rubra* possesses a variety of uses in the traditional system of medicine. Fresh aerial parts of *Basella rubra* contain triterpene oligoglycosides, basellasaponins A, B, C, and D.⁹ *B. rubra* contains Vitamins A, B, calcium and iron.¹⁰ It is used in urticaria, gonorrhoea, diuretic and as a cooling effect.¹¹ *Basella rubra* leaves have been reported as antiulcer activity.¹² The plant has previously been investigated for hypoglycaemic, antimicrobial, antifungal and for antiulcer activities.¹³ but there is no information regarding the intestinal anti-inflammatory activity. Hence the present study is designed to evaluate phytochemical and anti-inflammatory studies of methanol extract of leaf of *Basella rubra*.

MATERIALS AND METHODS

Collection & authentication of plant

The *Basella rubra* leaves were collected from Tirupathi in the month of October to November 2012 depending on its easy availability. The plant materials were

identified and authenticated (Herbarium. No. SPMVV/BT/BR1/2012) by Dr. N. Nagaraju, Assistant professor, Dept. of Botany & HOD of Biotechnology, Sri Venkateswara Arts College, Tirupati.

Preparation of plant material

The leaves were initially washed in tap water, then with distilled water. It was subjected to shade dry and further crushed to powder. The powder was extracted with methanol in soxhlet apparatus at 60°C and filtered to yield the extract. The extract was concentrated to dryness to get a solid mass. This was used for further study.

Phytochemical studies

The phytochemical studies were carried out as Palinisamy P *et al*¹⁴ with slight modifications. Methanol extract of leaves of *Basella rubra* was tested for identification of active constituents by qualitative tests.

Animals

Young adult male Wistar rats weighing 150-200 grams were used for this study are acclimatized to the laboratory conditions for two weeks prior to experimental use and maintained on standard laboratory rat feed and water. Rats were fasted for 12 hrs prior experiments, while allowing access to water throughout the experiment. The animals are divided into four groups, five animals in each group.

Group-1 Control, distilled water

Group-2 Received the standard drug Oxazolone only

Group-3 Received plant extract *Basella rubra* (500mg/kg) only,

Group-4 Received the both Oxazolone and *Basella rubra* extract (500mg/kg).

Oxazolone induced colitis

Oxazolone is the most widely used chemical for animal models of intestinal inflammation. Oxazolone method was followed as described by Wirtz S *et al.*¹⁵ On day 0 the skin of the rats in group 2 and 4 was treated with 150µl of Oxazolone or 150µl of vehicle control for presensitization. The Oxazolone presensitization solution is 4 parts acetone to 1 part of olive oil containing 3 % (wt /volume) Oxazolone. The vehicle control was four parts acetone to 1 part olive oil alone. After one week, rats were weighed and anesthetized, 100µl Oxazolone solution was given to the group 2 and group 4 rats by rectal administration. The Oxazolone solution was 1% Oxazolone mixed into a 50% ethanol solution. The vehicle control was 50% ethanol solution alone. Group 2 serves as control for Oxazolone induction (inflammation).

Group 3 and 4 received the oral dosage of *Basella rubra* extract prior and during the Oxazolone treatment. After 7 days of induction, rats were weighed, euthanized and colons were processed for pathological studies.

Histopathology

The colons were collected from four groups of rats. All colons were made into “Swiss rolls,” fixed in 10% formalin, and then paraffin-embedded. These Swiss rolls were used for histopathology sections for clear observation of colon structures. Sections were stained with H&E (Hematoxylin and Eosin). The degree of inflammation in the colon sections were evaluated by pathologist.

Quantifying inflammation

Slides were examined in a blind fashion by a trained pathologist. Inflammation was graded by extent (focal, multifocal, diffuse or extensive areas) and depth/penetration of inflammation (lamina

propria, into sub mucosa, into muscularis propria and into subserosa.) was assessed by the overall extent in the colonic tissue. Inflammation was given with a numerical value of 0-4. Where 0 is none observed and 4 is severe inflammation and / or ulceration / erosion.

RESULTS

Phytochemical screening of the extract

Phytochemical screening revealed that methanol extract of leaves of *Basella rubra* showed presence of alkaloids, terpenes, tannins, saponins, flavonoids and anthraquinones. These results were shown in table 1.

Changes in body weights of rats

The rats weights were measured and compared with control group rats. Significant changes in the body weights of rats were observed, it is shown in figure 1. There is an abundant decrease in the body weights of group 2 rats after the induction is due to inflammation in their colons. Dietary intake was low in group 2 rats that lead to the decrease in body weights and they became so dull and inactive during the experimental period. Whereas in group 4, rats treated with Oxazolone and of *B. rubra* (500mg/kg) extract during experimental period, thereby there is a recovery in body weights by having the good dietary intake and inflammation recovered to some extent. Rats in group 3 were continuously fed with dosage of *B. rubra* (500mg/kg) body weights were slightly increased at the end of experiment. No difference was found in the control rats before and after the dosage.

Morphological changes in colon structures and colon lengths

Rats weights and colons lengths were measured upon euthanasia. In control group rats have colon length of (17.3 cm). In oxazolone treated rats colons have the

inflammation with bulge structures and colon length shrink with stress and inflammation (15.9 cm). Rats fed with only *B. rubra* extract does not shown any morphological difference in their colons structures, lengths of the colons were also increased (18.0 cm). Rats treated with oxazolone and *B. rubra* extract has no inflammation in their colons and colon lengths were increased (16.9 cm) compared to group 2. These results were given in figure 2. This result were further confirmed by histopathology studies.

Histopathology of Colons

Examination of hematoxylin and eosin sections of normal colon structure was seen in control slide (Fig 3a) with closely packed nuclei, simple tubular colonic crypts with normal mucosal layer were observed. In (Fig 3b) wide areas of mucosal necrosis, with elongated, irregularly branched glands that contain epithelium with cytological and nuclear atypia including loss of differentiation (goblet cells), polarity, and enlarged nuclei were seen in Oxazolone treated group. Only plant extract treated group (Fig 3c) is similar to control slide with narrowly packed nuclei with normal mucosal layer. Rats treated with Oxazolone and *B. rubra* extract (Fig 3d) has recovery in their damage in mucosal layer and arrangement of nuclei is close compared to group 2. These results showing the activity of *B. rubra* on inflammation.

Inflammation score

The macroscopic inflammation score was given to the colons in different groups. Control group colons have no inflammation. In group 2, score was found to be significantly increased due to inflammation in colons by Oxazolone induction. Colons in group 3 has also no inflammation like control colons, it indicates the normal control colon structures. Group 4 colons

having the minimal inflammation. Here *Basella rubra* shows a recovery in inflammation, induced by Oxazolone. These results indicates that the group treated with Oxazolone and *B. rubra* extract has a significant lower inflammation score compared with the group treated with Oxazolone alone (Table 2). Inflammation was scored as follows: 0, no inflammation; 1, minimal; 2, mild; 3, moderate; 4, marked and severe.

DISCUSSION

The plant selection was based on its traditional claims and its folkloric usage for the treatment of various diseases. Screening for phytochemicals in plants is prime step in elucidating the pharmacological properties of a plant species.¹⁶ Alkaloids are known to exhibit anti-inflammatory, cytotoxic antibacterial, analgesic and anticancer activities.¹⁷ Flavonoids and tannins are phenolic compounds that are known to have antimicrobial, anti-inflammatory, cytotoxic, allergic, analgesic and antioxidant properties.¹⁸ Terpenoids are reported to have anti-inflammatory, anticancer, antimalarial and antibacterial properties.¹⁹ Phytochemical results of our study shows the presence of alkaloids, terpenes, tannins, saponins, flavonoids and anthraquinones. The result of phytochemicals is similar to previous report with an exception of the presence of alkaloid compounds in the same family member *B. alba* belongs to basellaceae.²⁰ Pre-clinical screening methods were used to evaluate the anti-inflammatory activity of new substances.²¹ In this study by use of Oxazolone (haptening agent), it is bound to a substance of high molecular tissue proteins that turns into antigen. That elicit an immunological responses for generation of IBD.²² As there is a severe inflammation in the colon, the food intake was decreased that results in decreased body weights as referred by Little JR *et al*.²³ Our results indicated that

methanolic extract of *B. rubra* (500 mg/kg b.w.) improving the body weights comparing to treated group. This activity is due to presence of various phytochemicals in the methanol extract of *B. rubra*.

Conclusion

From the obtained results, it can be concluded that the extract of *Basella rubra* shows a significant anti-inflammatory activity which was demonstrated in Oxazolone method. It can be concluded that the methanol extract of leaves of *B. rubra* have interesting anti-inflammatory property. Further investigations may be carried out to isolate the active constituent responsible for this activity.

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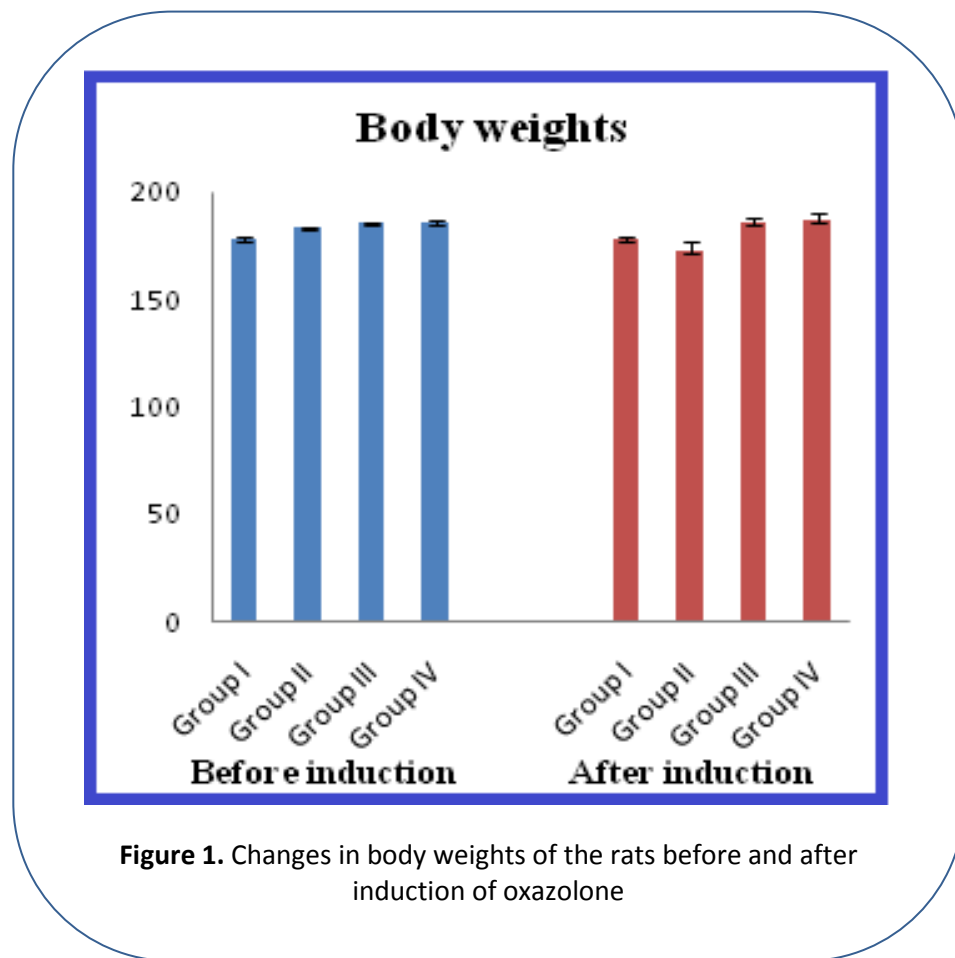
Table 1. Phytochemical components of methanol extract of *Basella rubra*

Phytochemical components	Leaf extract
Alkaloid	++
Terpene	+++
Tannin	+++
Saponins	+++
Flavonoids	+
Anthraquinone	+

+ =Present, --- = Absent

Table 2. Effect of *Basella rubra* on the prevention of inflammation in the colon induced by oxazolone

Groups	Inflammation score (0-5)	Number of animals
Group 1	0	5
Group 2	4	5
Group 3	0	5
Group 4	1	5



*Values are mean \pm S.D.

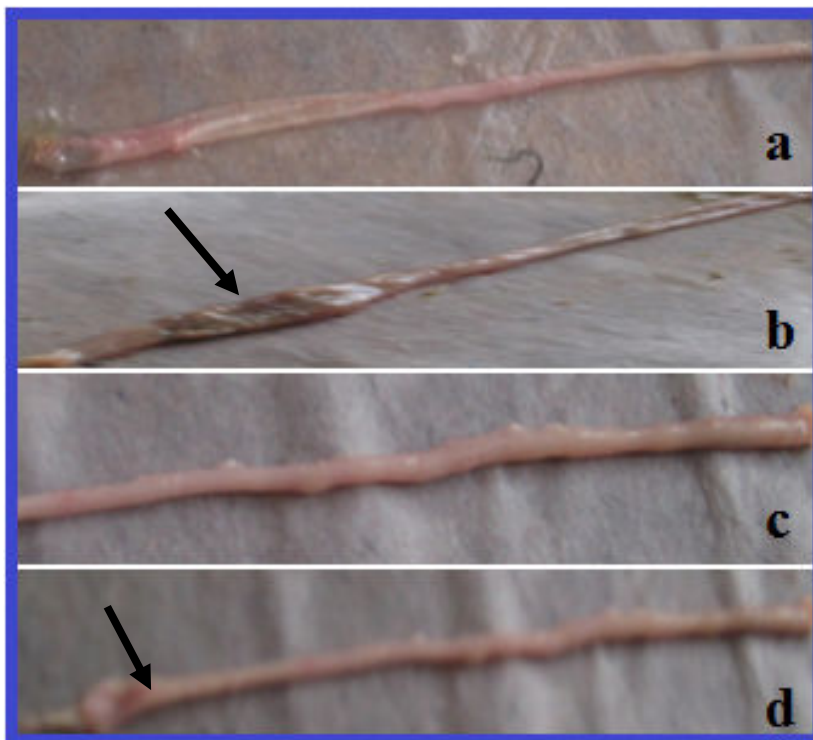


Figure 2 (i). Changes in colon in different groups. (a) Colon from control rats. (b) Colon from Oxazolone induced rats (arrow mark indicates inflammation in the colon). (c) Colon from only *B. rubra* extract (d) Colon from oxazolone and *B. rubra* (500mg/kg) treated rats (arrow mark indicates recovered inflammation in the colon)

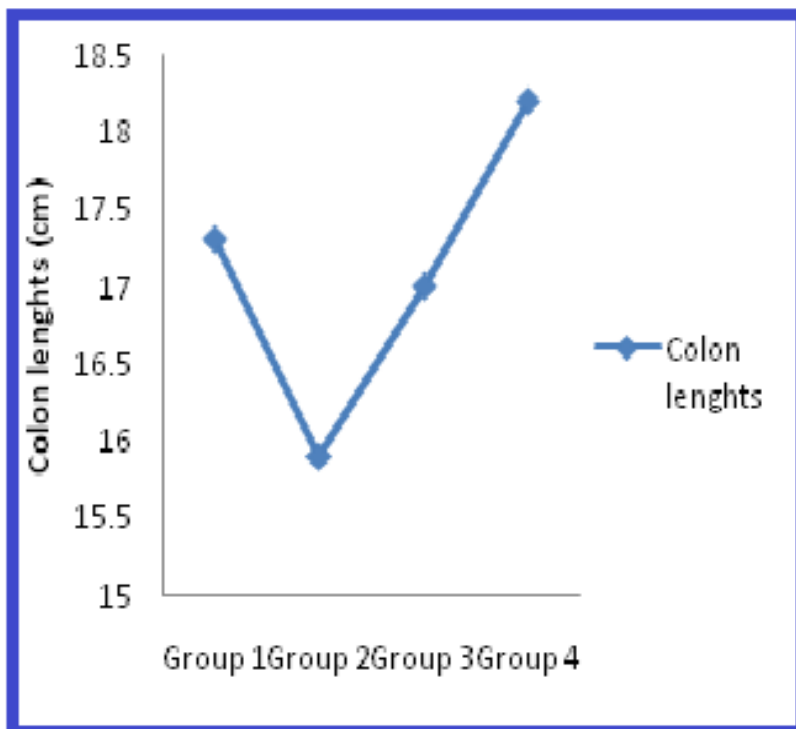


Figure 2 (ii). Representing colon lengths in different groups at the end of experiment

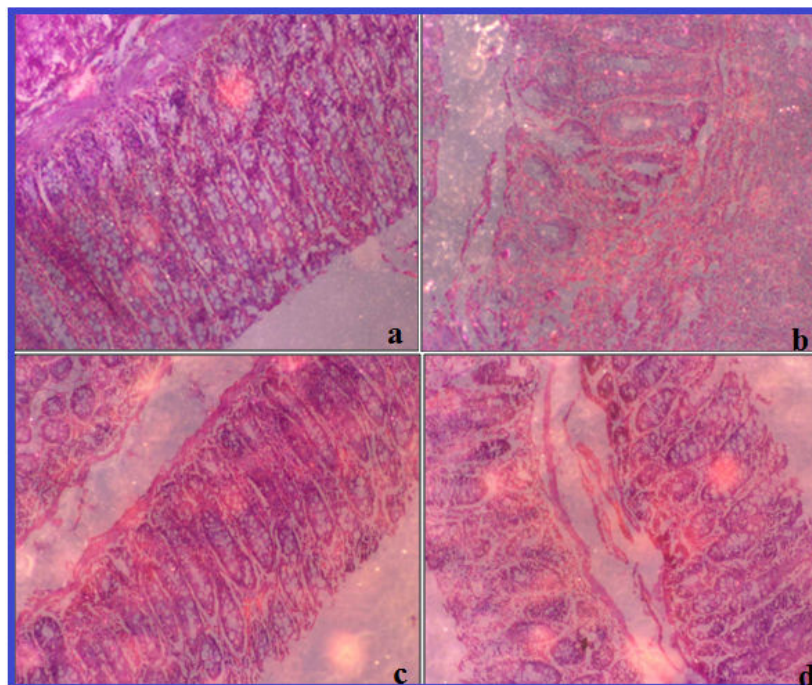


Figure 3. Showing the Hematoxylin and Eosin sections of the colons. (a) Colon from control group, (b) Colon from animals treated with Oxazolone, (c) Colon from animals treated with only *B. rubra* (500mg/kg), (d) Colon from animals treated with Oxazolone and *B. rubra* (500mg/kg)