



Evaluation of anti-inflammatory activity and anti-oxidant potential of portulaca quadrifida linn.

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Article History

Received: 19-10-2021

Revised: 08-11-2021

Accepted: 12-12-2021



Keywords: *Portulaca quadrifida* Linn. Mice, anti-inflammatory activity, Diclofenac.

Abstract

*Portulaca quadrifida*L. (PQ) having the phytochemicals like Alkaloids, flavonoids, Saponins, tannins, glycosides, carbohydrates, aminoacids, triterpenoids. The present study was performed to evaluate the antioxidant and anti-inflammatory activities by using acetic acid induced vascular permeability model in mice & acetic acid induced colitis in rats significantly. PQ (100 mg/kg, p.o.) presented a significant anti-inflammatory activity towards acetic acid induced vascular permeability model in mice in comparison to Diclofenac sodium(10 mg/kg, s.c.) and acetic acid induced colitis in rats in comparison to 5-ASA. Our findings suggest that, PQ contains potential antioxidant and anti-inflammatory compounds which will aid us to conduct bioactivity guided isolation & characterization of leading compounds in due course.

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<https://doi.org/10.37022/jiaps.v6i3.255>

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Introduction

Plants are one of the most important sources of medicine. Today the large number of drugs in use is derived from plants, such as morphine from *Papaver somniferum*, Aswagandha from *Withaniasomnifera*, Ephedrine from *Ephedra vulgaris*, Atropine from *Atropa belladonna*, Reserpine from *Rauwolfia serpentina* etc. The medicinal plants are rich in secondary metabolites (which are potential sources of drugs) and essential oils of therapeutic importance. The important advantages claimed for therapeutic uses of medicinal plants in various ailments are because of their safety, besides being economical, effective and their easy availability. Because of these advantages the medicinal plants have been widely used by the traditional medical practitioners in their day today practice. Plants have been used for health and medicinal purpose since thousand

years. They are one of the rich and important sources of medicine since human civilization. Now a day, it is preferred to use plant based medicines over synthetic medication for the treatment of different diseases because of their safety and cost effectiveness. Herbal medicines are particularly used by traditional practitioners since ancient times, inspite of their poor scientific data. Moreover continuous exposure to stressful conditions generates free radicals, which may overpower the inbuilt protective mechanisms and cause tissue damage. These are reports that plants possessing free radical scavenging activity are known to have organ protective effect. Many scientists are reported that, flowers and green leaves of the plant are useful as medicines [1, 2].

Natural antioxidants present in the plants scavenge harmful free radicals from our body. Free radical reactions have been implicated in the pathology of numerous diseases [3]. It is possible to reduce the risk of chronic diseases & prevent disease progression by either enhancing the body's natural antioxidant defences or by supplementing with proven dietary antioxidants [4]. Synthetic antioxidants like ascorbic acid, butylhydroxyanisole (BHA) and butylhydroxytoluene (BHT) are commonly used in foods have their potential health risks & toxicity. Thus the need for alternative sources of antioxidant is paramount & the search for natural

Antioxidant, especially of plant origin has received much attention [5]. Plant polyphenolic compounds, such as flavanoids are described as scavengers of ROS. Most sources of natural antioxidant originate from plant material. It has been also reported that reactive oxygen species (ROS) participate in the process of inflammation in various tissues [6]. In addition to their role in acute inflammation, several factors are recognized to contribute to the pathogenesis of inflammatory bowel disease (IBD) including an overgeneration of reactive oxygen species (ROS). Therefore, compounds that have scavenging activities toward these radicals may be expected to have therapeutic potentials for several inflammatory diseases [7, 8].

Most clinically important medicines belong to steroidal or non-steroidal anti-inflammatory chemical therapeutics for treatment of inflammation-related disease. Though these have potent activity, long term administration is required for treatment of chronic disease. Furthermore, these drugs have various and severe adverse effects. Therefore, naturally originated agents with very little side-effects are required to substitute clinical therapeutics [9].

Medicinal plants are believed to be an important source of new chemical substances with potential therapeutic effects. Plants of genus *Leucas* are widely used in traditional medicine to cure many diseases such as cough, cold, diarrhoea and inflammatory skin disorder. The genus *Leucas* comprises of about 2,500 species. The highest species diversity has been found in East Africa. In India 43 species are available [10].

Portulacaquadrifida Linn belongs to the family portulacaceae. [11]. It is a small diffused, succulent, annual herb found throughout the tropical parts of India. (param jyothiswamy .et al. 2010) [12]. Medicinally *Portulacaquadrifida* is used less widely but has some medicinal applications like "hypotensive and antidiabetic [13]. It is said to be useful in asthma, cough, urinary discharges, inflammations, and ulcers. A poultice of the plant is applied in abdominal complaints, erysipelas and haemorrhoids [14].

Portulacaquadrifida L. having the phytochemicals like Alkaloids, flavonoids, Saponins, tannins, glycosides, carbohydrates, amino acids, triterpenoids [15]. The alkaloid extracts obtained from medicinal plant species have multiplicity of host-mediated biological activities, including antimalarial, antimicrobial, antihyperglycemic, anti-inflammatory and pharmacological effects [16]. Alkaloids have alpha glucosidase inhibitory effects [17]. Which helps in antihyperglycemia. Saponins are glycosides with distinct characteristics and have been reported to possess a wide range of biological activities. Saponins and glycosides have antidiabetic activity [18]. Cherian S et al. work shows that a single dose of glycosides treatment decreased fasting blood glucose by 19% and improved glucose tolerance by 29%. The

Corresponding effects of glibenclamide were 25% and 66% respectively over the control values [19].

The main aim of the present study is to evaluate the anti-inflammatory activity of the hydro-alcoholic extract of *Portulacaquadrifida* L. which was not reported yet.

Materials and Methods

Plant Material

A small diffused, succulent, annual herb: *Portulacaquadrifida* Linn obtained in the vicinity were collected.

Animals

Swiss mice (20–25 g) and Wistar Albino rats (150-200) of both sexes were obtained & were maintained in a controlled environment at 22±2 °C and 55±10% humidity with 12 h light–dark cycle and fed with standard pellet food and water *ad libitum*. The experimental design & research plan along with animals handling and disposal procedure were placed before the institutional ethics committee.

Extract preparation

The aerial parts of the plant were shade dried for 10 weeks. After the plant material is completely dried they were powdered by using mixer. Fine powder was obtained by sieving. Finally they were stored in a dry container for the further process. Then the powder is extracted in a soxhlet apparatus with a solvent ethanol and it is pretreated with 80% ether twice to remove some coloured materials, oligosaccharides, and some small molecule materials. The organic solvent was volatilized and pre-treated dry powder was obtained, as described previously. The pre-treated dry powder was extracted with deionised water, while the temperature of the water bath ranged from 70-100 °C and was kept steady. The entire mixture is stirred during the entire extraction process. The extract obtained was evaporated to get a concentrated drug.

Preliminary chemical characterization of the extract *Leucas diffusa*

The extracts prepared were tested for the type of chemical constituents present by known qualitative tests.

Anti-inflammatory activity

Acetic acid induced vascular permeability in mice

Four groups (n=6) of swiss mice were fasted for 18h before the experiment. One group received low dose of HEPQ1 (500mg/kg) and other group received high dose of HEPQ 2 (1000mg/kg), third group received Diclofenac sodium (1000mg/kg). Control group received distilled water. Each animal were injected with freshly prepared 0.6% acetic acid in NaCl 0.9% solution. The volume injected should be 1ml/100g through intraperitoneal route. Then, 10mg/kg of 10% (w/v) Evans's blue was injected intravenously through the tail vein immediately after administration of acetic acid. After 30min of Evans blue injection, the animals were held by a flap of abdominal wall & the viscera irrigated with

distilled water over a petri dish. The dye leaking out into the peritoneal cavity was measured using UV spectra at 610nm.

Acetic acid induced colitis in Albino rats

Healthy albino rats, weighing 150–200 g, were used in the study and were divided into five groups (n=6) as follows:

Group A (normal control) received 1% CMC 10 ml/kg/day p.o.,

Group B (test) received HEPQ 1(500mg/kg/day p.o),

Group C (test) - HEPQ 2 (1000mg/kg/day p.o),

Group D (standard) -5- aminosalicyclic acid (5-ASA) 100 mg/kg/day p.o,

Group E (Toxic control) – no treatment.

All the animals were pre-treated with the respective drugs (volume of drug was kept constant at 5 ml/kg) for 5 days. On the fifth day, animals were fasted overnight and colitis was induced the next morning in Groups B, C and D, E by administration of 1 ml of 4% acetic acid solution. The administration should be done rectally using paediatric catheter under low dose of ether anaesthesia.

The rats were kept for 30 seconds in head-down position to prevent leakage. All the animals were sacrificed after 48 hours of colitis induction, by ether overdose. Abdomens were opened and colons were exposed. Distal 10 cm of colon was excised. Colon was opened by a longitudinal incision. Wash the mucosa with saline solution and mucosal injury was assessed macroscopically no damage (score 0); localised hyperaemia but no ulceration (score 1); linear ulcer without significant inflammation (score 2); linear ulcer with significant inflammation at one site (score 3); two or more sites of ulceration and inflammation (score 4) and two or more sites of ulceration and inflammation or one major site of inflammation and ulcer extending >1 cm along the length of colon (score 5). Disease activity indexes (DAI), degree of tissue oedema were also measured.

Histological analysis

Histological analysis was done by excising a 6–8 mm sample block of the inflamed colonic tissue from a region of grossly visible damage. Formalin-fixed tissue samples were embedded in paraffin and stained with haematoxyllin–eosin (HE).

Colonic tissues were scored for histological damage

- 0=intact tissue with no apparent damage;
- 1=damage limited to surface epithelium;
- 2=focal ulceration limited to mucosa;
- 3=focal, transmural inflammation and ulceration;
- 4=extensive transmural ulceration and inflammation bordered by normal mucosa;
- 5=extensive transmural ulceration and with inflammation in entire area.

Biochemical Assessments

Preparation of the sample

The proximal 5 cm of the dissected colon specimen was collected. The colonic samples were minced and homogenised using a Polytron homogenizer. Then, centrifuge the samples at 3000rpm for 20min. The supernatant was used for biochemical analysis of MPO, CAT & SOD.

Myeloperoxidase (MPO) activity

The minced colonic samples were homogenised in 10 ml of icecold 50 mM potassium phosphate buffer (pH 6) containing 0.5% hexadecyl trimethyl ammonium bromide (HETAB). The homogenates were then sonicated and centrifuged for 20 minutes at 12,000 rpm. From this, 0.1 ml of supernatant was collected and to this, 2.9 ml of 50 mM phosphate buffer containing 0.0005% H₂O₂ was added. The change in absorbance was measured spectrophotometrically at 460 nm. One unit of MPO activity is defined as the change in absorbance per minute at room temperature, in the final reaction. $MPO \text{ activity (U/g)} = X/\text{weight of the piece of tissue taken}$, Where $X=10 \times \text{change in absorbance per minute/volume of supernatant taken in the final reaction}$.

Malondialdehyde (MDA) level

0.75ml of rat serum was taken. To this, 3ml of the reagent (75mg of Thiobarbituric acid (TBA) was dissolved in 15% TCA, to this 2.08ml of 0.2N HCL was added, the volume was made up to 100 ml using 15% TCA) was added. The test tubes were kept in a boiling water bath for 15 minutes. They were cooled and centrifuged for 10 minutes at 10000rpm. Absorbance of the supernatant was read against the blank at 535nm. The results were expressed in nmol/ml of serum.

Evaluation of antioxidant status in colonic tissue

CATALASE estimation

Phosphate buffer (2.5 ml, pH 7.8) was added to the supernatant and incubated at 25°C for 30 minutes. Absorbance was measured at 240 nm spectrophotometrically. Hydrogen peroxide (650 µl) was added and change in absorbance was measured for 3 minutes. Values were expressed as µmol/min/mg of proteins.

SOD estimation

The colonic samples were mixed with 3.0ml of potassium phosphate buffer, centrifuged at 2000 rpm for 10 minutes and the supernatants were collected. To that add 0.2ml of NADH to the mixture and incubated at 30°C for 90 seconds and finally the reaction was arrested by the addition of 1.0ml of glacial acetic acid. Then add 4ml of n-butanol to the reaction mixture, allowed to stand for 10 minutes and centrifuged. The absorbance was measured at 560nm in a spectrophotometer.

sStatistical analysis

Results were expressed as mean±SD. Statistical analysis was performed by oneway analysis of variance (ANOVA) followed by the tukey's test for multiple comparisons. Differences were considered significant at P < 0.05.

RESULTS

Phytochemical evaluation

In the ethanolic extract the alkaloids, flavonoids, are rich whereas saponins, tannins, glycosides, carbohydrates, amino acids, triterpenoids are present at low levels. In water extract the alkaloids, flavonoids, saponins, and carbohydrates are rich and glycosides and amino acids are present at low levels and tannins and triterpenoids are absent.

Table 01: Phytoconstituents in ethanol and water

Phytoconstituents	Ethanol	Aqueous
Alkaloids	+++	+++
Flavonoids	+++	+++
Saponins	++	+++
Tannins	++	--
Glycosides	+	+
Carbohydrates	--+	+++
Amino acids	+	++
Triterpenoids	+	--

Anti-inflammatory activities

Acetic acid induced vascular permeability

As shown in Figure 3, the amount of dye passed across vessel in control group was 3.339±0.09 µg/g b.w as the dye was administered according to body weight. The extract (1000 mg/kg) reduced significantly (p<0.05; versus control group). The amount of dye retrieved in peritoneal fluid was 1.721±0.03 (25.91%) and 0.913±0.02 (60.66%) µg/g of mouse, respectively. For comparison, the vessel leakage of the dye in the diclofenac treated group was 0.726±0.02 µg/g of body weight (68.52%) (Table2, Figure 1).

Table.2: Acetic acid induced vascular permeability

Group	Dose (mg/kg, p.o.)	Amount of Evans blue dye	% inhibition
Normal	---	0.396±0.00	--
Control	--	3.339±0.09*	--
Diclofenac	10	0.726±0.02**	68.52
HEPQ-1	500	1.721±0.03**	25.91
HEPQ-2	1000	0.913±0.02**	60.66

Values expressed as mean±SEM (n=6). *P<0.05 when compared to normal control; **P<0.05 when to experimental control; ANOVA followed by Tukey's multiple comparison test.

Acetic acid induced vascular permeability

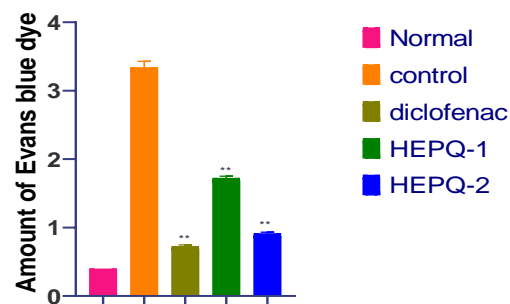


Fig.1: Acetic acid induced vascular permeability

Figure 1: Effect of the *Portulacaquadrifida L* hydroalcoholic extract on acetic acid induced increase of vascular permeability in mice. The amount of increase in vascular permeability was correlated to amount of dye measured in peritoneal cavity fluid. Values are expressed as mean ± sem (n=6). All groups are significant relative with each other: p< 0.05.

Acetic acid induced colitis in rats

As observed from this study, acetic acid administration to the experimental control group caused significant macroscopic ulcerations and inflammations (P<0.05) in rat colon along with significant mucosal injury microscopically (P<0.05), when compared to the normal control group. Also, there was significant derangement of biochemical parameters including tissue levels of MPO, MDA, CAT, SOD and serum MDA (P<0.05), indicating oxidative stress due to colon damage and colonic inflammation. Hydro alcoholic extract of *Portulacaquadrifida* has shown significant activity against experimentally induced IBD when compared to that of the experimental control (P<0.05) animals, with an improved picture of colon architecture both macroscopically as well as microscopically.

Tab.3: Acetic acid induced colitis in rats

Groups	Macroscopic score	DAI	Microscopic score
Normal	0.0±0.0	3±0.51	0±0
Colitis control	4.21±0.06*	7.16±1.01*	4.33±0.21*
Standard drug	1.11±0.03**	1.66±0.42**	1.33±0.21**
HEPQ-1	2.31±0.07**	3.16±1.66**	2.66±0.21**
HEPQ-2	2.05±0.02**	1.83±0.33**	2.16±0.16**

Values expressed as mean±SEM (n=6). *P<0.05 when compared to normal control; **P<0.05 when to experimental control; ANOVA followed by Tukey's multiple comparison test; DAI – Disease activity index.

Antioxidant activity

There is reduction of oxidative stress with significant improvement in tissue levels of CAT, SOD (P<0.05), showing its antioxidant potential. There is also significant improvement in the levels of MPO, showing its potential anti-inflammatory activity (P<0.05)

Tab.4: Antioxidant activity

Groups	Tissue MPO (U/g)	MPO inhibition %	Serum MDA (nmol/ml)	Tissue CAT ($\mu\text{mol}/\text{min}/\text{mg}$)	Tissue SOD (U/mg of proteins)
Normal	62.42 \pm 1.23	--	3.28 \pm 0.04	1.066 \pm 0.010	7.59 \pm 0.042
Colitis control	196.5 \pm 1.21*	--	5.58 \pm 0.11*	0.467 \pm 0.007*	2.94 \pm 0.020*
Standard drug	78.16 \pm 0.04**	60.19	3.06 \pm 0.01**	0.970 \pm 0.007**	5.15 \pm 0.014**
HEP Q-1	96.03 \pm 1.27**	51.12	2.41 \pm 0.02**	0.670 \pm 0.006**	5.21 \pm 0.002**
HEP Q-2	88.15 \pm 0.33**	55.12	2.27 \pm 0.01**	0.631 \pm 0.008**	5.36 \pm 0.016**

Values expressed as mean \pm SEM (n=6). *P<0.05 when compared to normal control; **P<0.05 when to experimental control; ANOVA followed by Tukey's multiple comparison test.

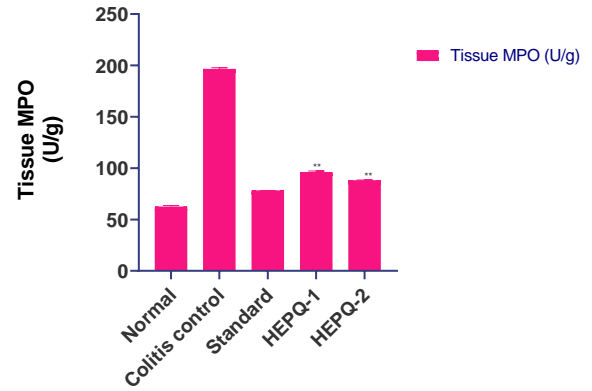
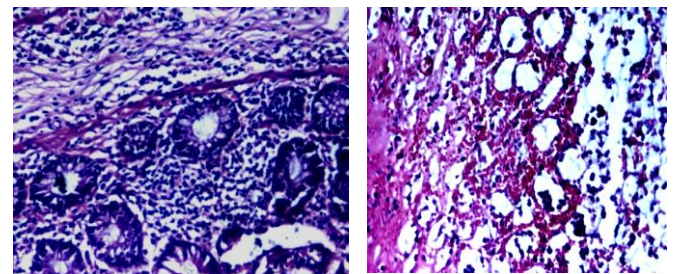


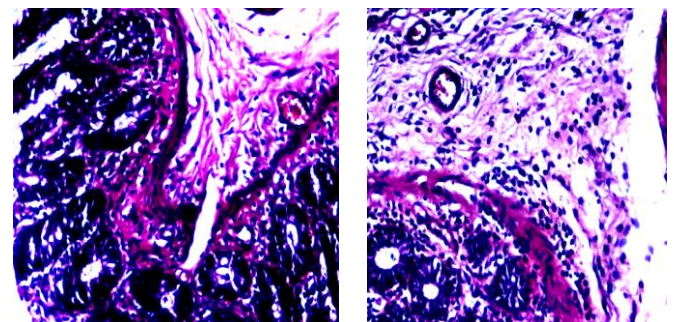
Fig.4: MPO, showing its potential anti-inflammatory activity

Histology

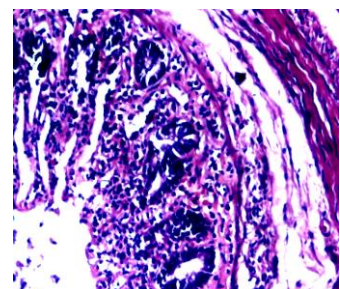
(Normal control) – Normal mucosal architecture
 (Experimental control)- Extensive necrosis with transmural infiltration



(Standard)- Near normalisation of architecture with mucosal infiltration only
 (HEPQ-1)- Focal ulceration limited to mucosa



(LDME-2)- Focal ulceration limited to mucosa



Effect of HEPQ On Induced Colitis

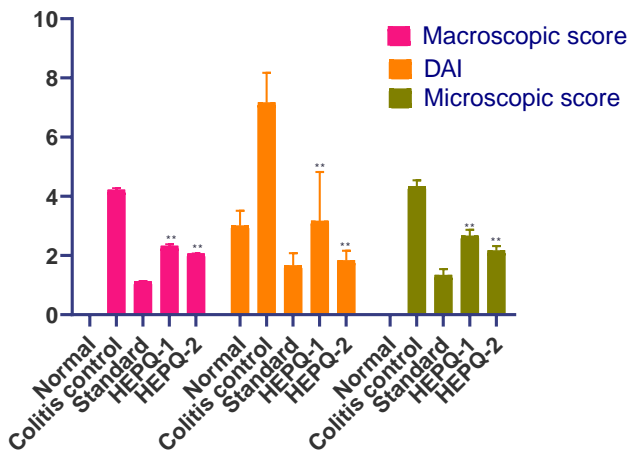


Fig.2: Effect of HEPQ on Inducer colitis

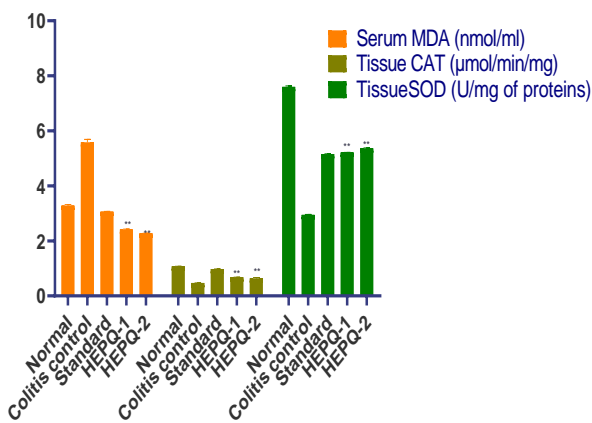


Fig.3: Tissue levels of CAT, SOD showing its antioxidant potential.

Discussion

Isolation of pure, pharmacologically active constituents from plants remains a long and tedious process. For this reason, it is necessary to have methods available which eliminate unnecessary separation procedures. Chemical screening is thus performed to allow localization and targeted isolation of new or useful constituents with potential activities. This procedure enables recognition of known metabolites in extracts or at the earliest stages of separation and is thus economically very important. Flavonoids, (a large group of naturally occurring plant polyphenolic compounds including flavones, flavonols, isoflavones, flavonones and chalcones), also known as nature's tender drugs, possess numerous biological and/or pharmacological activities. Recent reports of antiviral, anti-fungal, antioxidant, anti-inflammatory, antiallergenic, antithrombic, anticarcinogenic, hepatoprotective, and cytotoxic activities of flavonoids have generated interest in studies of flavonoid-containing plants. Flavonoids have demonstrated to exert beneficial effects on some diseases involving lipid peroxidation. The capability to interact with protein phosphorylation and the antioxidant, iron-chelating, and free radical scavenging activity may account for the wide pharmacological profile of flavonoids [75-78].

Antioxidants that scavenge free radicals play an important role in cardiovascular disease, aging, cancer, and inflammatory disorders [77]. In addition, these naturally occurring antioxidants can be formulated to give nutraceuticals, which can help to prevent oxidative damage from occurring in the body. The extract was screened for its potential antioxidant activities using tests such as hydroxyl radical-scavenging activity, reducing power activity, and hydrogen peroxide-scavenging activity. The *in-vitro* antioxidant assay showed *Portulacaquadrifida L. hydro-ethanolic extract* possesses potent antioxidant activity when compared with reference compound ascorbic acid. *Portulacaquadrifida L* could be useful for preparation of nutraceuticals as potent antioxidant to treat various human diseases and its complications. The crude hydroalcoholic extract of *Portulacaquadrifida L* was also tested for acute & chronic anti-inflammatory activity and it showed significant anti-inflammatory activity at a dose of 1000mg/kg. Vascular permeability change participates in pathophysiology of inflammation with leakage of vascular contents to interstitial tissue. That was assessed by the amount of Evans blue dye which extravasated to peritoneal fluid in acetic acid induced peritonitis in the mice. The extract of *Portulacaquadrifida L* reduced significantly vascular permeability. The extract contains chemical components which could interfere with the metabolism or the targets of released vascular active mediators. Acetic acid induced colitis model is similar to human ulcerative colitis in terms of histological features.

It affects the distal colon portion and induces non-transmural inflammation, massive necrosis of mucosal and submucosal layers, mucosal oedema, neutrophil infiltration of the mucosa and submucosal ulceration. The protonated form of the acid liberates protons within the intracellular space and causes massive intracellular acidification resulting in massive epithelial damage. Inflammation is the pathogenesis of IBD, and several pathways are associated with inflammatory response in IBD due to mucosal intestinal flora. The inflammatory response initiated by acetic acid includes activation of cyclooxygenase and lipoxygenase pathways. The results showed that hydroalcoholic extract of *Portulacaquadrifida L* has got a significant protective activity against experimental colitis in rats, as indicated by DAI, macroscopic, microscopic and biochemical evaluations.

Myeloperoxidase (MPO) is an enzyme mainly found in azurophilic granules of neutrophils. It can serve as a good marker of inflammation, tissue injury and neutrophil infiltration in gastrointestinal tissues. Pretreatment with *Portulacaquadrifida L* exhibits decrease in polymorphonuclear infiltration demonstrated by significant reduction in MPO activity. Oxidative damage may represent crucial pathogenic factor in IBD because intestinal inflammation is accompanied by increased production of reactive oxygen and nitrogen species.

MDA is considered as an important indicator of lipid peroxidation [79], which is found to be increased in rats treated with acetic acid. This might be due to lipid peroxidation. Rat pretreatment with *Portulacaquadrifida L* showed protection against lipid peroxidation characterised by significant decrease in MDA level.

Oxidative stress is believed to play a key role in the pathogenesis of IBD-related intestinal damage. Intestinal mucosal damage in the IBD is related to both increased free radical production and a low concentration of endogenous antioxidant defence. The antioxidant enzymes, mainly SOD, CAT are first line defensive enzymes against free radicals and ascorbic acid is also known to control oxidative damage [80]. In the present study it was observed that the hydroalcoholic extract of *Portulacaquadrifida L* significantly increase antioxidant parameters (CAT and SOD) in colitis induced rats.

Conclusion

The present study indicates that the hydroalcoholic extract of whole plant *Portulacaquadrifida L* have got profound antioxidant effect and showed significant anti-inflammatory activity at higher dose. This may be due to presence of flavanoids in the hydroalcoholic extract. Further purification, characterization of leading compounds is needed in due course.

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