INTRODUCTION

Ulcerative colitis (UC) is a chronic, inflammatory illness of the colon which affects the innermost lining of the intestines. The condition leads to inflammation, sores or ulcers, and bleeding in the gastric mucosal lining. Symptoms include a progressive relaxing of the stool, abdominal cramps, rectal bleeding, and loss of appetite that result in intestinal oxidative injury in UC patients [4]. Chronic inflammation, infection, or immune imbalance increases the production of ROS and impairs antioxidant defenses, resulting in intestinal oxidative injury in UC patients [4].

Punica granatum plant commonly known as the pomegranate is an attractive shrub or small tree growing 6–10 m high with multiple astringent qualities of the flower juice, peel, and tree bay is used to luxury diarrhea, dysentery, and duodenal parasites. The whole plant of P. granatum was collected in the months of September–November from the Ayurvedic Gardens of Banaras Hindu University, Varanasi. They remained reserved in the departmental animal house of the Institute of Medical Sciences, Banaras Hindu University, Varanasi. They remained reserved in the departmental animal house of the Institute of Medical Sciences, Banaras Hindu University, Varanasi. They remained reserved in the departmental animal house of the Institute of Medical Sciences, Banaras Hindu University, Varanasi.

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MATERIALS AND METHODS

Animals

Inbred Charles-Foster albino rats (160–180 g) and mice (25–30 g) of either sex were obtained with permission from the central animal house of the Institute of Medical Sciences, Banaras Hindu University, Varanasi. They remained reserved in the departmental animal house of the Institute of Medical Sciences, Banaras Hindu University, Varanasi. They remained reserved in the departmental animal house of the Institute of Medical Sciences, Banaras Hindu University, Varanasi. They remained reserved in the departmental animal house of the Institute of Medical Sciences, Banaras Hindu University, Varanasi.

Plant material and preparation of extract

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RESULTS

The study was undertaken to evaluate the healing effects of 50% ethanol extract of P. granatum peel (PGE) on acetic acid-induced colitis in rats. Optimal healing was observed by the administration of a 100 mg/kg dose of PGE extract. Effectiveness of the above-mentioned dosage of PGE, biochemical parameters namely antioxidants-superoxide dismutase and reduced glutathione were studied on 18 h fasting rats on the 15th day of the experiment.

Conclusion: PGE extract was found to be effective in healing mucosal damage due to colitis by controlling the infection and reducing the inflammation.

Keywords: Ulcerative colitis, Punica granatum peel extract, Acetic acid, Sulfasalazine, Antioxidants.
University. The peel was dried in shade and blended to form a fine powder and used. 50% ethanol-except of PGE was prepared by adding 200 g of dried fine powder of PGE in 1000 ml of ethanol (500 ml) and distilled water (500 ml) mixture. The mixture was shaken at regular intervals and the extract so obtained was filtered after a gap of 2 days. PGE so got each time remained mixed and later dried at 40°C in the incubator. The yield was about 20% (w/w). PGE stood stored at -20°C until further use.

Drug and chemicals
Sulfasalazine (TAB. SALAZAR 500 mg by Zydus Cadila), acetic acid, and all the other chemicals, and reagents that were used were of analytical grade.

Chemicals

Estimation of protein
a. Alkaline preparation - 50 ml Alkaline reagent + 0.5 ml of 4% Superoxide dismutase (SOD) Pot. Tartrate + 0.5 ml of 2% copper sulphate. (Alkaline reagent - 2 g of sodium bicarbonate + 100 ml of 0.1 N NaOH)

b. Phenol reagent - 5 ml of phenol + 5 ml of distilled water.

Estimation of antioxidants
SOD
a. Sodium pyrophosphate (Sigma) – 0.052 M sodium pyrophosphate was prepared by adding 1.16 g in 50 ml of distilled water and the pH was adjusted to 8.3

b. Phenazinemethosulphate (PMS) (Sigma) – 186 µmol of PMS prepared by adding 0.018 ml of 100 mM of PMS to 10 ml of distilled water. 100 mM of PMS prepared by adding 0.306 g in 10 ml of distilled water
c. Nitroblue Tetrazolium (NBT) (Sigma) – Prepared in a concentration of 300 µM by dissolving 2.754 mg of NBT in 10 ml of distilled water
d. NADH (Sigma) – 780 µM concentration of NADH was prepared by dissolving 1.11 mg in 20 ml of distilled water.

Reduced glutathione (GSH)
a. 0.4 M Tris buffer (Sigma) – Prepared by dissolving 4.84 g of Tris in a small amount of distilled water and adding 10 ml of 0.2 M sodium EDTA (EDTA) and making up to 100 ml with distilled water. Adjust the pH to 8.9 with 1 N HCl
b. 0.2M EDTA – 7.4 g of the sodium salt of EDTA (E. Merck) was dissolved in 100 ml of distilled water
c. 0.02 M EDTA – Take 10 ml of 0.2M EDTA and make up to 100 ml with distilled water
d. 0.1M DTNB – Dissolve 99 mg of DTNB (Hi-Media) in 25 ml of absolute methanol
e. 50% Trichloroacetic acid (TCA) – 50% solution was prepared by dissolving 10 g TCA in 20 ml of distilled water
f. GSH standard: Standard GSH was used for the estimation of the standard curve.

Induction of colitis and treatment protocol
Experimental colitis was produced by intracolonic administration of acetic acid (10%, 0.20 ml/100 g rat) given per rectally. [6]. A dose-response study was first undertaken with 50% ethanol extract of PGE (50, 100 and 200 mg/kg) when given orally for a period of 14 days after the induction of UC with acetic acid and on the 15th day of the experiment in 18 h fasting rats. The animals were sacrificed on the 15th day after the induction of UC with acetic acid and on the 15th day after the induction of UC with acetic acid. Antioxidant enzymes play an important role in healing and therefore levels of antioxidants such as SOD and reduced GSH were estimated in mucosal incubates from the normal, AA-induced colitis. Antioxidant enzymes play an important role in healing and therefore levels of antioxidants such as SOD and reduced GSH were estimated in mucosal incubates from the normal, AA-treated, and extracts treated AA-induced colitis.

Biochemical estimations

Estimation of protein
To estimate the protein content in the colonic mucosal homogenate, 95% ethanol was added to 0.1 ml of colonic mucosal homogenate (100 mg/ml), and was then centrifuged at 3000 rpm for exactly 5 min. The hurried so obtained was dissolved in 1 ml of 0.1N NaOH. 0.4 ml of the above example was taken in another test tube. 4 ml of alkaline reagent was then added to this test tube and kept for 10 min. Then, 0.4 ml of the phenol substance was added and again 10 min were allowed for color development. The absorbency was measured at 610 nm spectrophotometrically against blank prepared using distilled water. The protein content was estimated from the standard curve prepared with bovine albumin and has expressed as mg/g wet tissue.

Estimation of antioxidants
SOD
SOD was estimated by following the procedure of Kakkar and associates [1984]. n-Butyl alcohol was used to measure the inhibition of reduction of NBT to blue colored formazan in presence of PMS and NADH at 560 nm. To 0.4 ml of the homogenate add 1.2 ml of sodium pyrophosphate buffer (pH 8.3, 0.052 M), 0.1 ml of 186 µM of PMS, 0.3 µl 300 µM nitrobiotetrazolium, and 0.8 ml of distilled water to prepare a volume up to 3 ml including 0.2 ml of NADH (780 µM). NADH was used to initiate the reaction. After incubation at 30°C for 60 s, the reaction remained stopped by the adding of 1.0 ml of glacial acetic acid. 4.0 ml of n-butanol was added to the reaction mixture which was then mixed thoroughly after stirring well. The mixture was allowed to stand for 10 min, centrifuged and the butanol layer was taken out. The color intensity of the chromogen in the butanol was measured at 560 nm in a spectrophotometer against butanol. Control was selected to be a solution that was free of any enzymes. One unit of enzyme activity is defined as enzyme concentration required inhibiting the optical density at 560 nm of chromogen protection by 50% in one min under the assay conditions, and the results have been expressed as units (U) of SOD activity/g wet tissue.

Reduced GSH
About 0.5 ml of homogenate was mixed with 0.4 ml of distilled water and 0.1 ml of 50% TCA. The tubes were shaken intermittently for 10–15 min and centrifuged for 15 min at 3000 RPM. 0.6 ml of supernatant was mixed with 0.8 ml of 0.4 M Tris buffer and 0.02 ml of DTNB and the example was shaken. The absorbance was read within 5 min of the adding of DTNB at 412 nm against a reagent blank with no homogenate. The results were expressed as mmol/g-wet tissue and were calculated from the standard curve prepared using standard GSH.

Statistical analysis
Statistical comparison was performed using either unpaired “t” test or one-way analysis of variance and for multiple comparisons versus control group was done by Dunnett’s test. All statistical analysis was did using SPSS statistical version 16.0 software package (SPSS® Inc., USA). p<0.05 were considered statistically significant.

RESULTS
The effects of PGE extract (test extract, 100 mg/kg) and sulfasalazine (positive control, 100 mg/kg) on biochemical paradigms related to the inflammatory process and healing were estimated in mucosal incubates following induction of colitis by acetic acid. Antioxidant enzymes play an important role in healing and therefore levels of antioxidants such as SOD and reduced GSH were estimated in colonic mucosal homogenates from the normal, AA-treated, and extracts treated AA-induced colitis.

Effect on antioxidants- SOD and GSH
AA-treated animals showed a significant decrease in both SOD and GSH levels in the colonic mucosal incubates when expressed either as per g wet tissue weight or per mg protein compared to normal untreated rats. PGE (100 mg/kg) and SS (100 mg/kg) when given for 14 days after AA-induction of colitis reversed the above change both in SOD and GSH levels near to NS group (Table 1 and Fig. 1).
**Table 1: Effects of PGE, and SS action on AA-induced changes in free radicals (lipid peroxidation and nitric oxide) and antioxidants (superoxide dismutase and glutathione) in AA-induced ulcerative colitis in rat’s colon**

| Oral treatment (mg/kg, OD × 14 days) | Protein | Anti-oxidants | GSH |
|-------------------------------------|---------|---------------|-----|
|                                     | mg/g wet tissue | IU/g wet tissue | IU/mg protein | nmol/g wet tissue | nmol/mg protein |
| NS+CMC 1%                           | 57.0±6.2 | 22.1±1.83 | 0.37±0.05 | 154.7±152.6 | 27.6±2.75 |
| AA+CMC 1%                           | 51.2±2.98 | 8.3****±1.3 | 0.17****±0.02 | 775.4±29.6 | 15.4****±1.3 |
| AA+PGE 100%                         | 66.9±2.03 | 21.5±1.96 | 0.32±0.03 | 1800.3±197.46 | 27.0±2.99 |
| AA+SS 100%                          | 65.3±1.88 | 19.8±1.14 | 0.32±0.01 | 1944.7±98.93 | 30.0±2.17 |

Results are mean±SEM of 6 rats in each group. **<0.01, ***<0.001 compared to respective NS group (unpaired “t” test), >0.01, <0.001, compared to respective AA group (Statistical analysis was done by one way analysis of variance followed by Dunnett’s test for multiple comparisons). CMC: Carboxymethyl cellulose, SOD: Superoxide dismutase, GSH: Glutathione, PGE: Punica granatum peel.

Antioxidants and colonic microorganisms play an important role in tissue injury and healing which were affected in AA-induced colitis, and their reversal by PGE, do suggest a possible role of *P. granatum* in the treatment of colitis which could be due to their promotion effect on antioxidant activity. Further, PGE seemed to be safe and did not produce any lethal effect.

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**CONCLUSION**

The results of the present study revealed that 50% ethanolic extracts of PGE have significant healing activity in both AA-induced colitis rats.

The results of the present study with the PGE extract (test extract, 100 mg/kg) and sultasalazine (positive control, 100 mg/kg) on various physical and biochemical parameters of colonic damage and inflammation induced by AA do indicate the effective healing effects of PGE.

**DISCUSSION**

Acetic acid-induced colitis has been reported to look like human inflammatory bowel disease in footings of prolonged colonic inflammation and serves as a useful model to investigate the role of free radicals and antioxidants in the pathophysiology of colitis [8,9]. Our results with intracolonic management of acetic acid showed an increase in inflammation and ulceration in the mucosa of rat’s colon. Estimation of antioxidants such as SOD and reduced GSH in colonic mucosal tissues has also been relevant because these antioxidants hasten the process of healing by destroying the free radicals. The significant alteration in the antioxidant profile may be attributed to impairing healing in immunocompromised rats. Our studies on the SOD, and GSH status revealed that PGE possessed significant antioxidant activity which would help to prevent oxidative damage and promote the healing process and its effect was comparable with the sultasalazine-treated group. The above effects may be attributed to the anti-inflammatory, antiinocceptive and antulcer properties of PGE. The presence of anti-inflammatory activity in PGE may be attributed to other pharmacologically active constituents such as ellagic acid, ellagitannins, puninic acid flavonoids, anthocyanidins, anthocyanins, and estrogenic flavonoids and flavones isolated earlier [10,11]. Flavonoids are most commonly known for their antioxidant activity. Thus, PGE could have flavonols and flavones isolated earlier [10,11]. Flavonoids are most commonly known for their antioxidant activity. Thus, PGE could have a potential role in the therapy of various inflammatory conditions including UC.

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