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Protective Effect of*Jaft Extract on* Acetic Acid Induced UlcerativeColitis in Rats

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ABSTRACT

Ulcerative colitis (UC) is chronic inflammatory diseases which typically affect colon and rectum. UC is a dysregulated immune response due to high levels of free radicals and inflammatory mediators. The aim of this work was to evaluate possible protective effects of aqueous extract of external layer of oak fruit (*Jaft*) in acetic acid- induced UC in rats. Forty two male wistar rats were randomly divided into six groups with seven rats in each. Negative control group was treated orally and intrarectally with saline. Positive control group was received intrarectally acetic acid in saline for three days as acetic acid induced ulcerative colitis group. Group I were received Jaft extract orally for four days before and three successive days during induction of colitis. Group II were received Jaft extract orally for only three successive days during induction of colitis. Group III: treated intraperotineal (i.p.) with L- Arginine in a dose of 0.5 g/kg body weight/day for four days before and three successive days during induction of colitis (as standard 1).Group IV: treated intraperotineal (i.p.) with L-Arginine in a dose of 0.5 g/kg body weight/day for three successive days during induction of colitis (as standard 2). After last dose of acetic acid on day 8, animals were anesthetized by diethyl ether and blood collection by heart puncture for assay of antioxidants and inflammatory markers in serum. Acetic acid administration decreased GSH, while MDA and NO concentrations were increased in the serum of rats compared to normal control group. Administration of *Jaft* extract ameliorated the biochemical alteration due to acetic acid induced ulcerative colitis by increasing GSH and decreasing MDA, NO in the serum of rats. This study concluded that use of L-arginine and *Jaft* extract could be helpful in preventing the relapse of UC disease due to their possible antioxidant and anti-inflammatory effects.

Keywords: *Jaft* extract, Ulcerative colitis, Free radicals, inflammatory mediator, Yasuj

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INTRODUCTION

Inflammatory bowel disease (IBD) is a chronic inflammatory disorder withidiopathic etiology whichis characterized by spontaneously relapsing. IBD be divided into two groups, ulcerative colitis (UC) and Crohn's disease (CD).Ulcerative colitis is usually involves alone mucosa of the colon and rectum .Whereas, Crohn's disease is an inflammation of the gastrointestinal tract which affect the entire tract [1]. Prevalence of IBD is rising in Western European and North American. It is estimated 10-70 per 100,000 populations to be affected by UC [2, 3].

UC occur anytime in life but often in the people 15-35 years old. Itaffects both sex men and women equally [1].Patient with UC usually present with diarrhea, blood in stool, abdominal pain, cramping,fever,fatigue, weight loss and in severe cases shortening of the colon and weight loss [4, 5].The exact cause of UC is undetermined but related to complex interaction between immune system and genetic and environmental factors. Changes in dietary and intestinal bacteria play a pathogenesis role [5].Genome scan have present susceptibility genes for UC disease on chromosome 1 and 4[4].

Imbalance in the generation of free radicals and antioxidant component can be important in the pathogenesis of UC. Reactive oxygen species (ROS)activated the oxidative stress gene which is important in progression in UC. Neutrophils and macrophages infiltration is the important specification in UC.Neutrophils activation in the mucous tissue of intestinal produce oxygen reactive species including the superoxide ions (O_2-), hydroxyl radical (OH^o) and (H_2O_2). These factors cause lipid peroxidation and mucosalpermeability [6, 7, 8].

In UC inflammatory cytokines like IL-1β, IL-6, IL- 8and TNF-α released from mononuclear cell (macrophages).They are responsible for production of reactive oxygen species (ROS).High level of ROS caused destruction of intestinal epithelial layer and therefor lead to experimental colitis [1].Recent studies demonstrated No as a proinflammatorymediator isassociated with dysregulation of immune system response and therefore initiation of inflammation in ulcerative colitis. Indeedby the interaction of NO with ROS was produced peroxynitritewhich can be important in tissue destruction in colitis[9].

These products cause impairment in cell membrane function via lipid peroxidation in UC which monitored by MDA as a lipid peroxidation marker.Free radicals (superoxide, hydroxyl radicals and H2O2) are controlled by different cellular enzymes including superoxide dismutase (SOD), catalase (CAT) and non-enzymes as reduced glutathione (GSH).It is not required in the diet and is synthesized in cells from amino acids. Glutathione has antioxidant properties and is as a reducing agent. In cells, glutathione is maintained in the reduced form by the enzyme glutathione reductase[10].

Corticosteroids (dexamethasone),immunomodulators(azathioprine) and aminosalicylates are available for treating UC.In most cases, these drugs present adverse reactions and relapse of disease. A large number of medicinal plants have been reported which exhibit potential antiulceractivities (antiulcerative colitis).Treatment with natural products produce fewer side effects [11].

The internal layer of the oak fruit (*Quercus brantti*) is known as *Jaft* which is distributed in some parts of Iran especially in the northern and central parts.*Jaft* extract is traditionally used to treat inflammation,gastropathies,diarrhea,burnand cancer. Mechanisms action of this plant is due to anti-inflammatory and antioxidant properties. The antioxidant and anti-lipid peroxidation activity of the *Quercus brantti*) may be due to the presence of polyphenol and tannin compounds which can justify the protective effects of this plant[12].The aim of this study was to evaluate possible protective effects of Jaft extract in acetic acid induced ulcerative colitis.

MATERIAL AND METHOD

Collection and Extraction of Plant Material:

Fruits of the oak tree (*Quercus brantti*) were collected from the mountains of Yasuj, Iran, in January2014. Samples were submitted in the Herbal Medicinal Research Center, Yasuj University of MedicalSciences. After drying in the room temperature, extraction was carriedout indistilled water by the

maceration method for 24 h. Then the extracts was collected andfiltered through Whatman No.1 filter paper, and concentrated using a rotary evaporator (Heideolph model 4000; Germany). The dried extract was stored in the refrigerator at -20^{0C} for experimental use.

Experimental animals :

Forty two male Wistar rats (n=42), weighing 180- 250 grams were obtainedfrom animal Care Centre, Shiraz,Iran.Animals were kept in polypropylene cages , fed on standard pellet diet and allowed to adaptation to the laboratorystandard condition for one week before the experiment. Theywere maintained in a controlled temperature of 22 ± 2°C, 50% ± 20% humidity, and 12 h light/dark cycle. Experimental study was carried out according to the National Institute of Health Guidelines for animal care [13].

Induction of UC:

Colitis was induced in rats using the technique of intracolonic instillation of 2 ml (4% v/v) acetic acid solution in saline once daily .A catheter size 6F was inserted into the colon via the anus approximately the splenic flexure (8 cm from the anus) . Acetic acid was spread in the colon by administration of 2 ml air before taking out the catheter. After colitis induction, the animals were observed for three days. On day four, the animals were sacrificed [13].

Experimental protocol :

Animals were randomly divided into 6 groups; each group included seven rats as following: Negativecontrol group: treated orally and intrarectally with saline. Positive control group: received intrarectally 2 ml of 4% acetic acid in saline daily for three successive days (acetic acid induced –ulcerative colitis group).Group Ⅰ: treated with *Jaft* extract orally by gavage tube in a dose of 0.5 g/kg body weight/day for 4 days before and three successive days during induction of colitis using acetic acid (treat 1).

Group Ⅱ: treated with *Jaft* extract orally by gavage tube in a dose of 0.5 g/kg body weight/day for alone three successive days during induction of colitis using acetic acid (treat 2).GroupⅢ: treated intraperotineal (i.p.) with L- Arginine in a dose of 0.5 g/kg body weight/day for 4 days before and three successive days during induction of colitis using acetic acid (as standard 1).GroupIV: treated intraperotineal (i.p.) with L- Arginine alone in a dose of 0.5 g/kg body weight/day [18] alone three successive days during induction of colitis using acetic acid (as standard 2).

BIOCHEMICAL ASSESSMENTS

Malondialdehyde (MDA) assay:

MDA is a product of lipid peroxidation was assayed in serum by the method of Hoyland. In this method,500 µlserumswere added to 2 ml of MDA reagent (375 mg, TBA+15gr, TCA dissolvedcompletely in 0.25 M Hcl). The mixture was heated for 15 min in boiling water bath,then was cooled in ice and centrifuged at 2000g for 5min. Supernatant that contain MDA was measured at absorbance 535 nm by spectrophotometer against reagent blank(Pharmacia LKB.Novaspec II,Germany) [14].

Reduced Glutathione (GSH) assay:

Reduced Glutathione (GSH) levels of serum were determined by Ellman's reagent by the method of Moron [15].

Nitric oxide radical (NO°) assay:

NO Level was assayed according to Griess reagent method based on nitrite concentration by colorimetric. Briefly; 100 µL of serum was added to 100 µLGriess reagent (mixture of 1% sulfanilamide in 2.5% orthophosphoric acid and 0.1% N-(naphthyl) ethylenediamine in distilled water). Then was kept at room

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temperature for10 min, absorbance was measured at 540 nm byspectrophotometer (Pharmacia LKB. Novaspec II, Germany)[16].

Statistical analysis:

Statistical analysis was carried out using the SPSS statistics software ver.20 by one way analysis of variance (ANOVA) followed by Tukey's multiple comparison tests. Values are expressed as Mean±SD. p <0.05 were considered significant.

RESULTS

MDA and NO concentration in serum were significantly (P< 0.05) increased in UC group as compared to control group. Treatment with *Jaft* extract significantly decreased levels of MDA and NO as compared to UC group (Figure 1, 2). GSH level in UC group was significantly (P < 0.05) decrease when compared to control group. Treatment with 500 mg/kg per day of *jaft*extract, for one week significantly (P < 0.05) improved GSH concentration as compared to UC group (Figure 3).There was no significant difference between treated 1 and 2 and also standard 1 and 2.Treated 1 and standard 1 were preventive practice.No significantly difference were observed between in standards and Jaft extracts inMDA , No and GSH tests, however values in standard groups were more better than that Jaft extracts .

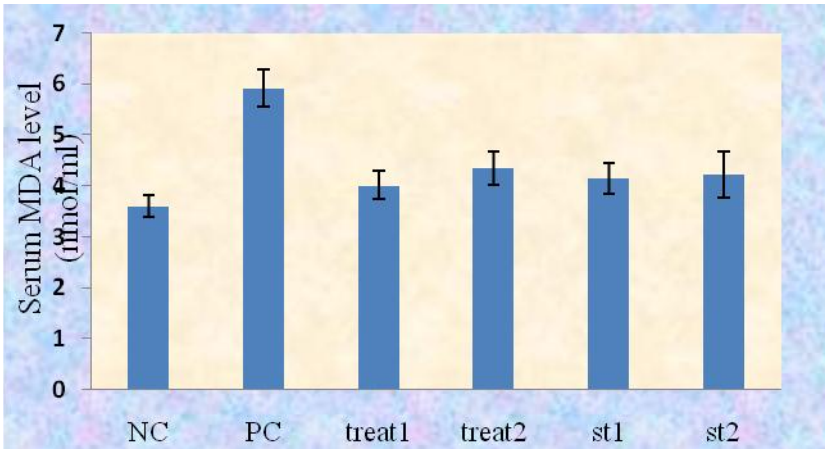

Figure 1. Effect of *jaft extract on* acetic acid-induced Ulcerative Colitis on malondialdehyde (MDA) NC=negative control, pC = Ulcerative Colitis, Treat1 and 2 = Jaft extract at dose 500 mg / kg body weight/day , St 1 and 2 = Arginine at dose 500mg/kg body weight/day)

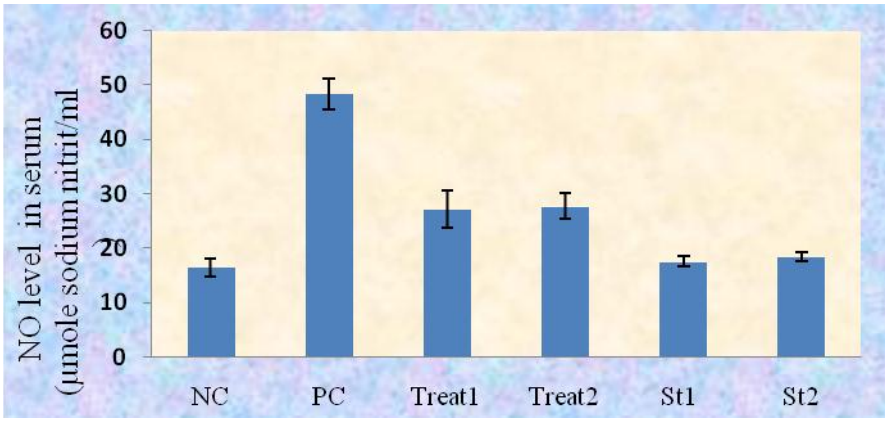

Figure2. Effect of *Jaft extract* on acetic acid-induced ulcerative colitis on nitric oxide NC= negative control, pC = Ulcerative Colitis, Treat1 and 2 = Jaft extract at dose 500 mg / kg body weight/day , St 1 and 2 = Arginine at dose 500mg/kg body weight/day

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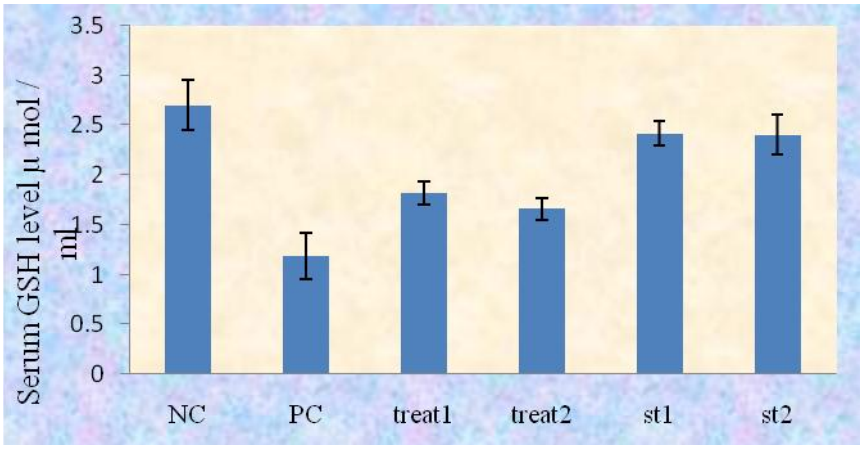

Figure3. Effect of *Jaft extract on* acetic acid-induced ulcerative colitis on glutathione (GSH)
NC= negative control, pC = Ulcerative Colitis, Treat1 and 2 = Jaft extract at dose 500 mg / kg body weight/day , St 1 and 2 = Arginine at dose 500mg/kg body weight/day)

DISCUSSION

Inflammatory bowel disease (IBD) is chronic relapsing disease characterized by inflammatory mediators and dysregulated immune response resulting in tissue damage and neutrophilsinfiltration. The exact cause of UC is unknown but a lot of genetics, immunology and environmental factors arerelated with IBD [1].

Induction of UC in rats using acetic acid is standard method to produce an experimental model of ulcerative colitis similar to human UCwhich showed increase in free radical, inflammation and ulceration. In addition, free radical can induce lipid peroxidationthat show by elevated level of MDA [17]. It has been shown that serum NOand MDA concentration increased and GSH decreased in experimental colitis model.

GSH as a non-enzymatic antioxidant is one enzymatic defense systems against free radicals generation which inhibits oxidative damage [10]. In present study the serum GSH levels in treated with *Jaft* extract groups increased compared to positive control group. Intrarectal administration of acetic acid leddecreasing of serum GSH levelin the positive controlgroup. In the present study a significant increase of MDA, NO and a decrease of GSH in serum of acetic acid induced UC as compared to the control group was seen.

Quercusbrantti exhibited pharmacological potential such as antioxidant and anti-lipid peroxidation activities in pervious experiments. Natural compounds present in plants, including tannins and polyphenol are as antioxidant agents. The using of natural product for treatment of disease is a new alternate. This study demonstrates protective potential aqueous extract of *Jaft.* Protective effects of extract has been attributed to the inhibition of oxygen reactive species (ROS).In the case of acetic acid-induced colitis, the inflammation triggers the generation of free radicals in the tissue which destroy intestinal mucosa.

CONCLUSION

This research demonstrated the protective effects of *Jaft* extract which can prevent the relapse and damaged of UC. This protection may relate to the antioxidant property and anti-inflammatory effects of *Jaft* extract.

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