EFFECT OF POMEGRANATE AND APPLE JUICE ON BODY WEIGHT, BLOOD CHEMISTRY AND COLON HISTOLOGY IN RATS WITH ULCERATIVE COLITIS INDUCED BY ACETIC ACID

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Abstract

Ulcerative colitis (UC) is one of the chronic inflammatory bowel diseases (IBD) that causes inflammation of colon mucosa and submucosa. This study aimed to investigate the effect of Pomegranate juice (PJ) and Apple juice (AJ) on body weight, blood chemistry and colon histology in rats with acetic acid-induced UC. Thirty-five mature rats were distributed into five groups (n=7 rats). Group 1 was kept negative control, group 2 was positive control with acetic acid-induced UC and groups 3, 4 and 5 with UC and orally given PJ, AJ and their combination for 6 weeks, respectively. Feed intake (FI), body weight gain (BWG %) and feed efficiency ratio (FER) were determined. Blood samples were collected for determination of serum aspartate aminotransferase (AST), alanine aminotransferase (ALT), uric acid (UA), creatinine (Cr.), total cholesterol (TC) and triglycerides (TG). Proinflammatory cytokines interleukin 1 beta (IL1β), IL6 and IL8 were measured. Glutathione peroxidase (GPx), superoxide dismutase (SOD) and catalase (CAT) were estimated. In colon homogenate, glutathione (GSH), malondialdehyde (MDA) and lactate dehydrogenase (LDH) were measured. Histopathological examination of colon was also performed. The results revealed that oral administration of combination of PJ and AJ to rats with UC significantly decreased body weight and serum levels of AST, ALT, UA, Cr., TC, TG and proinflammatory cytokines IL1β, IL6 and IL8. Combination of PJ and AJ increased serum GPx, SOD and CAT. It increased GSH, but decreased MDA and LDH in colon tissue homogenate. Histopathological examination of the colon showed that PJ and AJ together ameliorated inflammatory lesions of the colon in rats with UC. Therefore, drinking of PJ and AJ may be useful for patients who suffer from UC.

Keywords: Pomegranate, Apple, Ulcerative colitis, Body weight, Biochemistry, Cytokines, Histopathology

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1. INTRODUCTION

Ulcerative colitis (UC) is an idiopathic inflammatory bowel disease (IBD) that causes irritation, inflammation, damage, and ulcers in the mucosa of the large intestine and rectum. Multiple factors, such as genetic background, environmental and luminal factors, use acetaminophen for pain relief, and overactive immune response, have been suggested to contribute to pathogenesis of UC **(Ng et. al., 2013).** It has been suggested that these factors cause an excessive enteric immune response to gut flora or nutritional antigensis **(Katsanos and Papadakis, 2017).** UC is one of two major forms of inflammatory bowel disease (IBD) and is characterized by mucosal inflammation initiating in the rectum and extending proximally in the colon in a continuous fashion. By contrast, inflammation in Crohn's disease (CD), the other type of IBD, demonstrates patchy lesions that are potentially scattered anywhere in the gastrointestinal tract **(Magro, 2017).** Patients with UC may suffer from abdominal pain, bloody diarrhea, anemia, weight loss and blood or pus in bowel movements. Treatment of UC include drugs, change diets or surgery. Aminosalicylates, corticosteroids and immunosuppressant drugs can be used to reduce inflammation. Fresh or Canned fruits as apples, pomegranate, bananas, cantaloupe, and watermelon are good diets for colon inflammation.

Pomegranate (Punica granatum L) is a fruit- obtained from deciduous tree in the family Lythracaeae. Pomegranate juice has a wide range of health benefits including anti-inflammatory **(Martin et. al., 2013 and Rahimi et. al., 2020)**; antioxidant **(Gil et. al., 2000 and Balmus et. al., 2016)**; anti-obesity **(Lie et. al. 2007)**; anticancer **(Sharma et. al., 2006)** and hypochlosterolemic activities **(Esmillzadeh et. al.,**

2006). Pomegranate juice has been shown to possess an antidiabetic **(Saxema and Vikram, 2004)** and antihypertensive **(Sahebkar et. al., 2017)** effects.

Apple is a fleshy round red or yellow or green fruit of the family Rosaceae. The fruits are rich in fibers, anti-oxidants, vitamins and minerals. Apple fruits and juices showed powerful anti-oxidant **(Boyer and Liu, 2004)** and antiproliferative (Wolfe et. al., 2003) activities. Apple Cider vinegar decreased lipid peroxidation **(Abdulrauf et. al., 2018).** Whole apples or juice lowered cholesterol level in healthy volunteers (Raven-Haven et. al., 2013). The concentrate of apple extract reduced experimental colitis induced by acetic acid in rats **(Pastrelo et. al., 2017).**

The present study aimed to investigate the effect of Pomegranate juice and Apple juice on body weight, blood chemistry and histology of colon in rats with ulcerative colitis.

2. MATERIAL AND METHODS

- **Materials:**

➢ **Acetic acid (AA):**

Acetic acid is a simple monocarboxylic acid containing two carbon atoms. The molecular chemical formula of acetic acid is: CH3COOH. It was obtained as 5% solution (V/V) backed in 500 ml bottles from El-Gomhoriya Pharmaceutical Company, Cairo, Egypt. It is a liquid substance that can induce of ulcerative colitis in rats.

➢ **Basal diet:**

Basal diet was prepared according to the method of **Reeves et. al., (1993).** It is consisted of 20 % protein (casein), 10 % carbohydrate, 4.7% fat (corn oil), 2% choline chloride, 1% vitamin mixture, 3.5 % salt mixture and 5% fibers. The remainder was corn starch up to 100 %.

➢ **Rats:**

Thirty-five rats of Sprague Dawley strain weighing about 225± 5 g body weight and 8 months age were used in this study. Rats were purchased from the Agriculture Research Center, Ministry of Agriculture, Giza, Egypt. Rats were fed on basal diet and water was freely provided.

- **Methods:**

Induction of ulcerative colitis:

Rats were administered via intrarectal instillation of acetic acid (AA) at 24-hr intervals for 7 days with a plastic catheter. 0.5 ml of 3 % AA solution was instilled into the anus when each rat was maintained at inclined up position where the tail raised up and the head dropped down for 15 minutes to induce ulcerative colitis as reported by **Wang et. al., (2019).**

➢ **Experiment and rats:**

Thirty-five Sprague Dawley rats were housed in metallic cages with dry and clean wood shavings at room temperature (25±3 °C), 50% ± 5% humidity with a 12 hr dark/light cycle. Rats were adapted for one week on AIN-93 basal diet and freely allowed to water. The experiment was carried out according to the guidelines of the Institutional Animal Care and Use Committee, (IACUC), Faculty of Veterinary Medicine, Cairo University. Rats were distributed into five groups as follows: group1was kept negative (normal) control; group2: was positive control and AA was installed into the rectum while the tail was hold and the head was declined down to induce ulcerative colitis (UC). Groups 3, 4 and 5 were with induced UC and received orally 1ml/ rat of pomegranate juice, apple juice and their combination for 6 weeks, respectively. The rats were weighed at beginning and end of the dietary period. Daily feed intake (FI) was recorded day after day throughout the experimental period (8 weeks). Body weight gain % (BWG %) was calculated. Determination of body weight gain percent and feed efficiency ratio (FER) were assessed according to the method described by **Chapman et. al., (1959)** using the following equations:

BWG % = Final weight (g) – Initial weight (g) / Initial weight (g) ×100

Feed efficiency ratio (FER) = Daily weight gain (g) / Daily Feed consumed (g)

➢ **Blood collection:**

At the end of the experimental period (4 weeks), the rats were fasted overnight and anesthetized by sodium pentobarbital (Nesdonal®). Blood samples were collected from orbital plexus of eye and centrifuged at 10000 rpm for 15 minutes to obtain clear serum for biochemical analysis. Part of dissected colons was taken out for preparation of homogenates. The other part was kept in 10% neutral buffered formalin solution then processed for histopathological examination under light microscope.

➢ **Blood chemistry:**

After anesthesia of rats, the blood was withdrawn from orbital plexus of eye. Blood samples were collected for determination of activities of serum aspartate aminotransferase (AST) and alanine aminotransferase (ALT) according to the method of **Bergmeyer et. al., (1978).** Serum uric acid and creatinine levels were estimated as described by **Lorentz and Brendt (1967)** and **Agbafor et. al., (2015)**, respectively. Serum total cholesterol (TC) was calorimetrically determined according to **Allain et al., (1974)** and triglycerides (TG) according to **Wahlefeld (1974).** Interleukin 1 beta (IL1β), IL6 and IL8 were estimated according to **Sutton et. al., (2006).** Serum glutathione peroxidase (GPx), superoxide dismutase (SOD) and catalase (CAT) antioxidant enzymes were determined using a spectrophotometer according to **Paglia and Valentine (1967)**, **Nishikimi et. al., (1972)** and **Aebi (1984)**, respectively.

➢ **Preparation of colon homogenates:**

One gram of the colon was collected from each rat, washed in ice-cooled 0.9% NaCl solution and homogenized in ice-cooled 10 ml of 1.15 % potassium chloride solution in 50 mMol potassium phosphate buffer solution (pH 7.4) to get 10% (W/V) homogenate. Colon homogenates were centrifuged at 9000 rpm for 15 minute and the supernatant was utilized to determine reduced glutathione (GSH) **(Jollow et. al., 1974)**; malondialdehyde (MDA), a marker of lipid peroxidation, **(Ohkawa et. al., 1979)**, and lactate dehydrogenase (LDH) **(Hendrich and Hule, 1967).**

➢ **Histopathology:**

A part of the dissected colon was rinsed with saline solution and preserved in formalin solution then trimmed, washed and dehydrated in ascending grades of alcohol (70% to 100%). The colon tissue was sectioned and stained with Heamatoxylin and Eosin (H&E) then examined microscopically according to Bancroft and Stevens (1977).

- **STATISTICAL ANALYSIS:**

Data were expressed as means ± SEM and statistical analysis was carried using one-way ANOVA test with a computerized SPSS program. Significance was considered at $P < 0.05$ **(Snedecor and Cochran, 1986).**

3. RESULTS

Oral administration of PJ, AJ and their combination to rats with acetic acid-induced UC significantly ($P < 0.05$) decreased daily feed intake (FI), body weight gain% (BWG %) and feed efficiency ratio (FER) as compared to the positive control group **(Table 1).**

The results revealed that in the positive control group, there was a significant ($P < 0.05$) increase in both serum levels of AST and ALT enzymes as compared to the negative control group. Administration of PJ, AJ and their combination significantly ($P < 0.05$ decreased serum levels of AST and ALT as compared to the positive control group **(Table 2).**

There were significant increases in both uric acid and creatinine serum levels in the positive control group as compared to the negative control group. Administration of PJ, AJ and their combination significantly ($P < 0.05$) decreased serum levels of both uric acid and creatinine compared to the positive control group as reported in **Table (3).**

Table (1): Effect of Pomegranate juice (PJ) and Apple (AJ) and their combination on food intake (FI), BWG (%) and feed efficiency ratio (FER) in rats with ulcerative colitis induced by acetic acid (AA) (n=7 rats).

Parameters Groups	FI (g/day)	BWG (%)	FER
Normal control	22.0	10.00 ± 0.56^{a}	0.454 ± 0.001^{a}
AA	20.0	4.50 ± 0.41^{bc}	0.225 ± 0.002^{d}
AA+PJ	17.0	4.22 ± 0.20^{b}	0.248 ± 0.001^{c}
AA+AJ	16.9	4.11 ± 0.25^{c}	0.243 ± 0.002^{c}
AA+PJ+AJ	15.3	3.66 ± 0.52^{d}	0.239 ± 0.003^{b}

Means± SE with different superscript letters in the same column are significant at ($P < 0.05$).

Table (2): Effect of Pomegranate juice (PJ) and Apple (AJ) and their combination on liver enzymes aspartate aminotransferase (AST) and alanine aminotransferase (ALT) of rats with ulcerative colitis induce by acetic acid (AA) (n=7 rats).

Parameters Groups	AST (U/L)	ALT (U/L)
Normal control	39.57 ± 1.37^{e}	8.68 ± 0.66^{e}
AA	84.28 ± 2.57^{a}	30.57 ± 1.08^{a}
AA+PJ	48.17 ± 2.23^{b}	18.00 ± 0.53^{b}
AA+AJ	43.22 ± 1.78^{c}	15.14 ± 0.91^{c}
AA+PJ+AJ	40.71 ± 1.99^{d}	12.14 ± 0.79^{d}

Means± SE with different superscript letters in the same column are significant at ($P < 0.05$).

Table (3): Effect of Pomegranate juice (PJ) and Apple (AJ) and their combination on serum uric acid (UA) and creatinine (Cr.) serum levels of rats with experimentally-induced ulcerative colitis by acetic acid (AA). (n=7rats)

Parameters Groups	UA mg/dL	Cr. mg/dL
Normal control	34.14 ± 0.26^{e}	0.73 ± 0.07^{e}
AA	65.42 ± 1.88^{a}	2.52 ± 1.23^{a}
AA+PJ	50.71 ± 1.58^{b}	1.44 ± 1.26^{b}
AA+AJ	37.14 ± 12.63^{c}	1.24 ± 0.23^{c}
AA+PJ+AJ	35.42 ± 1.23^{d}	1.13 ± 0.30^{c}

Means± SE with different superscript letters in the same column are significant at ($P < 0.05$)

The results showed that serum levels of total cholesterol (TC) and triglycerides (TG) were significantly ($P < 0.05$) decreased by oral administration of PJ, AJ and their combination to rats with acetic acid-induced ulcerative colitis as compared to the positive control **(Table 4).**

Table (4): Effect of Pomegranate juice (PJ) and Apple (AJ) and their combination on Serum total cholesterol (TC) and triglyceride (TG) in rats with experimentally-induced ulcerative colitis by acetic acid (AA). (n=7 rats).

Parameters Groups	TC mg/dL	TG mg/dL
Normal control	34.14±0.26^{e}	140.0±2.07^{e}
AA	65.42±1.88^{a}	190.0±3.23^{a}
AA+PJ	50.71±1.58^{b}	166.0±2.26^{b}
AA+AJ	37.14±12.63^{c}	154.0±1.23^{c}
AA+PJ+AJ	35.42±1.23^{d}	151.0±2.30^{c}

Means± SE with different superscript letters in the same column are significant at ($P < 0.05$).

As shown in **Table (5)**, the rats with acetic acid-induced UC in the positive control group had significant ($P < 0.05$) increases of Interleukin-1 beta (IL-1β), IL6 and IL-8 as compared to the negative control group. Oral administration of PJ, AJ and their combination to rats with acetic acid-induced ulcerative colitis significantly ($P < 0.05$) decreased IL-1β, IL6 and IL-8 as compared to the positive control group.

Table (5): Effect of Pomegranate juice (PJ) and Apple (AJ) and their combination on rats with ulcerative colitis induced by acetic acid (AA) (n=7 rats).

Parameters Groups	IL1β (pg/ml)	IL6 (pg/ml)	IL8 (pg/ml)
Normal control	54.28±1.20^{d}	57.08±1.20^{d}	79.57±2.63^{d}
AA	87.57±2.39^{a}	70.16±1.39^{a}	88.14±1.52^{a}
AA+PJ	64.42±3.80^{b}	58.42±1.80^{b}	79.71±1.91^{b}
AA+AJ	44.00±4.76^{c}	55.00±1.76^{c}	64.80±2.74^{c}
AA+PJ+AJ	42.42±2.99^{c}	45.42±1.19^{c}	63.14±1.18^{c}

Means± SE with different superscript letters in the same column are significant at ($P < 0.05$).

Table (6) shows that oral administration of PJ, AJ and their combination to rats with acetic acid-induced ulcerative colitis significantly ($P < 0.05$) increased serum glutathione peroxidase superoxide (GPx), superoxide dismutase (SOD) and catalase (CAT) antioxidant enzymes as compared to the positive control group.

Table (6): Effect of administration of PJ, AJ and their combination to rats with acetic acid-induced ulcerative colitis significantly ($P < 0.05$) on serum glutathione peroxidase (GPx), superoxide dismutase (SOD) and catalase (CAT) antioxidant enzymes in rats with ulcerative colitis induced by acetic acid (AA) (n=7 rats).

Parameters Groups	GSH (nmol/mg protein)	MDA (nmol/mg protein)	LDH (nmol/mg protein)
Normal control	45.12±0.22^{a}	57.57±0.36^{e}	14.17±0.49^{b}
AA	29.85±0.34^{e}	76.14±1.88^{a}	18.28±0.52^{a}
AA+PJ	54.71±0.28^{d}	70.72±1.98^{b}	12.00±0.72^{c}
AA+AJ	59.14±0.80^{c}	68.85±1.56^{c}	10.71±0.28^{d}
AA+PJ+AJ	62.28±0.42^{b}	60.71±0.92^{d}	9.0.28±0.42^{e}

Means with different superscript letters in the same column are significant at ($P < 0.05$).

As recorded in **Table (7)**, the results indicated that administration of PJ, AJ and their combination to rats with acetic acid-induced UC increased reduced glutathione (GSH), but decreased malondialdehyde (MDA) and lactate dehydrogenase (LDH) in colon tissue as compared to the positive control group.

Table (7): Effect of administration of PJ, AJ and their combination to rats on colon tissue increased glutathione (GSH) and decreased malondialdehyde (MDA) and lactate dehydrogenase enzyme (LDH) in rats with ulcerative colitis induced by acetic acid (AA) (n=7 rats)

Parameters Groups	GPx (mg/dL)	SOD (mg/dL)	CAT (mg/dL)
Normal control	88.12±0.33^{a}	78.56±0.06^{a}	19.05±0.46^{a}
AA	65.85±0.24^{d}	49.42±1.13^{d}	10.0±1.12^{d}
AA+PJ	80.71±0.18^{c}	57.14±1.26^{c}	12.94±0.63^{b}
AA+AJ	82.14±0.16^{cb}	58.94±0.63^{cb}	13.55±0.63^{c}
AA+PJ+AJ	83.02±0.22^{b}	59.14±0.40^{b}	14.77±0.43^{bc}

Data are expressed as mean ± SD. *p< 0.05= significant

Several variables obtained at baseline were analyzed for the Means with different superscript letters in the same column are significant at (P < 0.05).

- **Histopathology:**

The histopathological findings of rat colon were illustrated in **Fig. (2 a-f)**

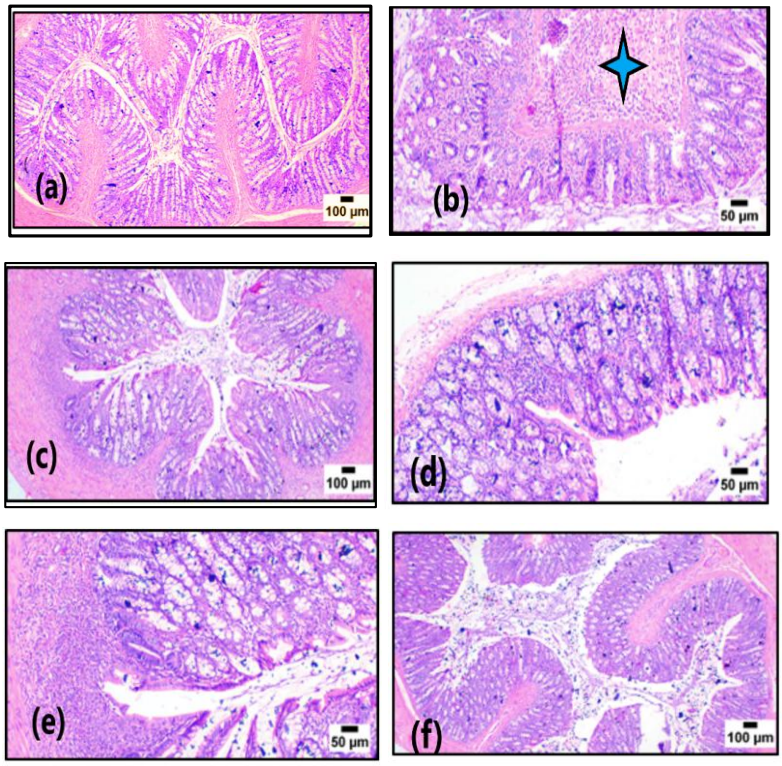

Fig. (2): Photomicrograph of rat colon of the different groups: (a): Normal control group showing normal histological structure of mucosa and submucosa of colon. (b) Positive control group showing heavy inflammatory cells infiltration (blue star) in the lamina propria and submucosa. (c) Positive control group showing decrease mucous exudation in the lumen with multifocal inflammatory areas in the submucosal layer. (d) Group orally given PJ for 6 weeks showing small foci of inflammatory cells aggregations in the lamina propria. (e) Group received orally AJ for 6 weeks showing few foci of inflammatory cells aggregations in the lamina propria. (f) Group orally given combination of PJ and AJ showing apparently normal histological archticture of colon mucosa and submucosa.

4. DISCUSSION

The aim of this study was to investigate the effect of oral administration of PJ, AJ and their combination to rats with UC on body weight, blood chemistry and histological picture of colon. The results revealed that PJ, AJ and their combination decreased feed intake (FI), body weight gain (BWG) and feed efficiency ratio (FER) causing weight loss in rats. These findings were in agreement with those reported by **Al-Moraie et. al., (2013)** who concluded that hypercholesterolemic rats fed on Pomegranate leaves powder at 1%, 3% and 5% significantly (P<0.05) decreased body weight gain (BWG), feed Intake (FI) and feed efficiency ratio (FER). **Soliman et. al., (2022)** found that feeding Pomegranate peel to overweight rats caused weight loss. The previous authors concluded that Pomegranate peel can be added to diets of people who suffer from overweight. the present results cleared that administration AJ to rats with UC showed that diet supplementation with Apple pomace (AP) and Apple juice concentrate (AC) caused body weight loss **(Gorinstein et. al., 2005 and Cho et. al., 2013).** The current results revealed that administration of PJ and AJ increased activities of serum antioxidant enzymes in rats with acetic acid-induced UC. These findings were similar to those of **Aviram et. al., (2000)** and **El-Mansi and Al-kahtani, (2019)** who concluded that pomegranate peel extract exerted cardioameliorative impacts due to its unique anti-oxidative and anti-inflammatory properties and suppressed the inflammatory responses and oxidative stress in gut of rats with acetic acid-induced UC. AJ administration to rats with UC induced antioxidant effect **(Balmus et. al., 2016).** Concerning liver functions, it was found that

that administration of PJ and AJ to rats with UC significantly reduced serum levels of AST and ALT enzymes. This was partially similar to that reported by **Pirmadah et. al., (2020)** in patients who mentioned that intake of PJ and AJ might positively affect liver function in diseased patients. Administration of PJ and AJ of rats with UC also significantly ($P<0.05$) decreased serum concentrations of UA and Cr. These results partially agreed with those recorded by **Primarizky et. al., (2016)** who found that the administration of pomegranate extracts in the treatment of nephrotoxicity was effective to maintain normal serum uric acid of the rats. Administration of PJ and AJ to rats with UC significantly decreased levels of proinflammatory cytokines Interleukin-1 beta (IL-1β), Interleukin- 6 (IL6) and Interleukin-8 (IL-8) in rats with UC. These findings were partially similar to those obtained by **Abd-elraman et. al., (2019)** who reported that Pomegranate extract induced a significant ($P<0.05$) decrease in serum Interleukin-1 beta (IL-1β) in rats with acetic acid –induced UC. Regarding antioxidant enzymes, oral administration of PJ and AJ of rats with UC increased serum GPx, SOD and CAT antioxidant enzymes. These findings agreed with those of **Aviram et. al., (2000)** who concluded that PJ and AJ produced an antioxidant effects. Oral administration of PJ and AJ decreased serum TC and TG in rats with UC. These findings were partially similar to that reported by **Sadeghipour et. al., (2014)** who found that PJ and peel induced lipid lowering effect in rats fed high fat diet. Histopathological examination of colons showed that administration of PJ and AJ to rats with UC ameliorated inflammatory cells infiltration. These findings were similar to those obtained by **Abdulrauf et. al., (2018)** and **Abd-elrahman et. al., (2019)** who concluded that administration of PJ and AJ to rats with UC mitigated inflammatory cells infiltration of colon.

5. CONCLUSION

It can be concluded that PJ juice and A J when given to rats with acetic acid-induced UC produces weight loss, improves liver and kidney functions, reduces proinflammatory cytokines, increases antioxidant enzymes and protects colon against UC. Therefore, drinking of PJ juice and AJ may be beneficial to patients who suffer from UC and oxidative stress.

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