Anti-inflammatory effects of *Citrus sinensis* L., *Citrus paradisi* L. and their combinations

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Abstract: Citrus bioflavonoids embrace a wide group of phenolic compounds effecting the production and scavenging of reactive oxygen species and the processes relating free radical-mediated injury. Keeping in view of the antioxidant and anti-inflammatory properties of *Citrus sinensis* and *Citrus paradisi*, present study was undertaken to explore the effects of *C. sinensis* (orange juice) and *C. paradisi* (grapefruit juice) at three different doses alone and their two combinations with the objective to examine the effects of these compounds in an experimental model of rat colitis induced by trinitrobenzenesulphonic acid (TNBS). Hence biochemical parameters e.g. myeloperoxidase, alkaline phosphatase, C-reactive protein (CRP) and glutathione were assessed. Data entry and analysis was accomplished by Statistical Package for the Social Sciences version 17 and was presented as mean ± S.E.M with 95% confidence interval. Present result shows that these juices, mainly *C. paradisi*, may be efficacious for the management of inflammatory bowel disease. In acute colitis model, *C. paradise* encouraged a decrease in the extension of the lesion escorted by a decrease in the occurrence of diarrhea and reinstatement of the glutathione content. Related effects were produced by the administration of *C. sinensis*, which also prevented the myeloperoxidase and alkaline phosphatase actions in acute intestinal inflammatory process. The effect of the citrus juices on the inflammatory process may be associated to their antioxidant and anti-inflammatory properties, as revealed in present investigation. The favorable effects exerted were demonstrated both by histological and biochemical changes and were related with a progress in the colonic oxidative status.

Keywords: Inflammatory bowel disease, trinitrobenzene sulfonic acid, Grape fruit, orange.

INTRODUCTION

Crohn's disease and Ulcerative colitis are chronic inflammatory bowel disease with recurring and natural remitting condition of the elementary tract. It is possibly associated to an unusual aggravated immune reaction to mild stimuli, which is not appropriately negated by the feedback system causing mucosal response to luminal factors down-regulation; although it's accurate etiology is unknown (Fiocchi, 1998). Irritable bowel disease (IBD) is described by an enhanced and increased numbers of the productions and discharge of diversity for pro-inflammatory mediators, such as reactive oxygen and nitrogen metabolites, eicosanoids, platelet-activating factor and cytokines, and other inflammatory processes causing excessive tissue injury that affect mucosal integrity (Katz *et al*. 1999; Podolsky and Fiocchi 2000). These inflammatory mediators induce biosynthesis and release of other such compounds resulting in propagation of the inflammatory response. A precise management of IBD is not accessible yet, however the best approach to efficiently reduce the aggravated immune response that illustrates IBD could be to obstruct with manifold phase of the inflammatory flow, rather a sole drug treatment (Kho *et al*., 2001). The treatments currently in practice for managing human IBD include 5-aminosalicylic acid derivatives and local or systemic glucocorticoids (Travis and Jewel 1994; Bratts and Linden 1996). However, serious side effect restrict the use of these drugs (Stein and Hanauer 2000; Irving *et al*., 2007). Thus based on these reasons, it is most important to explore a new drug treatment for IBD, which is safe and effective. Citrus fruit and juices have been considered a valued part of a healthy and nourishing diet, it is now well established that some of the nutrients in citrus juice encourage health and provide shield from chronic disease. More lately, the role of bioactive non-nutrient constituents known as phytochemicals has received growing attention (WHO 2003). Over 60 types of flavonoids have been identified in Citrus fruits distributed in the five different classes i.e. flavones, flavanones, flavonols, flavans and anthocyanins (Tripoli *et al*., 2007). Citrus flavanones are present in the glycoside or aglycone form, most important flavanones among aglycones are naringenin and hesperetin, while among the glycoside are neohesperidosides and rutinosides. Flavonoids have many biological activities e.g. antioxidant, anti-inflammatory, anti-atherosclerosis, anti-mutagenic, antiproliferative and antidepressant (Wilcox *et al*., 1999; Breinholt *et al*., 2000; Garai and Adlercreutz 2004; Yi *et al*., 2008; Hou *et al*., 2010; Ohlsson *et al*., 2010).

This study was conducted on fresh juices of two fruits, *Citrus sinensis* (orange) and *Citrus paradisi* (grapefruit)

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belonging to Rutaceae family. Grapefruit contains phytochemicals including limonoids and lycopene. It is also an exceptional source of vitamin C, dietary fiber, vitamin A, potassium, folic acid and vitamin B_5 (Mateljan 2006). It also contains high levels of iron, calcium and other minerals. Pink and red varieties of grapefruits are rich in beta carotene, more in fiber and less in calories. They possess protective plant chemicals like phenolic acid, limonoids, terpenes, monoterpenes and bioflavonoids, which protect from cancer and heart disease. The major bioflavonoid in grapefruit is naringin that gives grapefruit juice bitter taste (Armando *et al*., 1998; Giovannucci *et al*., 2002). Naringin exerts diverse pharmacological effects like antioxidant activity, lipid-lowering effect (Gorinstein *et al*., 2006), anti-carcinogenic activity (Armando *et al*., 1998) and blockade of specific cytochrome P450 enzymes including CYP3A4 and CYP1A2 (Gao *et al*., 2006). Grapefruit enhance appetite and is employed for its digestive, stomachic, antiseptic and diuretic properties (Herbal Medicine 2000).

Orange being a rich source of nutrients has also been known for a number of health and nutritional benefits. Orange contains nearly two hundred phytonutrients and flavonoids, which have shown activity against different types of cancers. They have been also described to have strong anti-inflammatory and anti-oxidant properties and prevent bone loss (Chiba *et al*. 2003; Gao *et al*., 2006; Peluso 2006; Liu *et al*., 2012). The essential oils in orange juice (*C. sinensis*) contain many constituents, including monoterpenes and sesquiterpenes with d-limonene as a major constituent (Graciela *et al*., 2003). Orange juice has been reported for cholesterol lowering effect in animal models (Kurowska *et al*., 2000) as well as humans (Roza *et al*., 2007). Its anti-inflammatory role in different disease is also well documented (Ghanim *et al*., 2010; Buscemi *et al*., 2012). Narirutin or Naringenin 7-O-rutinoside is another important flavonoid present abundantly in orange juice (Sawalha*et al*. 2009). It is absorbed well and shows good bioavailability (Manach *et al*. 2003). It is also shown to possess anti-inflammatory (Funaguchi *et al*., 2007; Ha *et al*., 2012), anti-allergic and anti-asthmatic effects (Funaguchi *et al*., 2007; Rogerio 2010). Orange and grape fruit are both rich in phytochemical, flavonoids and vitamins, which revealed to have strong anti-inflammatory and antioxidant properties (Chiba *et al*., 2003; Gao *et al*., 2006; Peluso 2006; Liu *et al*., 2012).

Many natural products possessing biological actions associated to their capacity to hinder enzymes and/or their antioxidant properties in relation to flavonoids, have been demonstrated to reduce the immune response (Middleton 2000). Thus in the treatment of IBD these actions could also make their consideration as suitable and effective drugs. As a matter of fact, earlier investigations have revealed the potency of some of these compounds, inclusive of hesperidin, quercitrin, rutoside, diosmin and morin, in the trinitrobenzene sulphonic acid (TNBS) model of rat colitis (Sanchez De Medina *et al*., 1996; Cruz *et al*., 1998; Crespo *et al*., 1999; Galvez *et al*., 2001a). Despite the fact that the act of mechanism of these flavonoids is not clearly recognized, a typical peculiarity in all cases is their capability to enhance the colonic oxidative stress that describes the intestinal inflammatory condition, most likely identified with their well-known cancer preventing and/or free radical eliminating properties ascribed to flavonoids. Supplementary mechanisms may also help in their favorable impact as morin demonstrated down regulation of mediators implicated in the intestinal inflammatory reaction e.g. cytokines and nitric oxide (Galvez *et al*., 2001b), according to latest report an early down regulation of the inflammatory flow can raise the positive effects of flavonoids on TNBS chronic colitis, which is related with improvement of the disorder in hydro-electrolytic transport (Sanchez De Medina *et al*., 2002).

METHODS

Animals

Adult male Wister rats having mean body weight of 220±10 grams were used in present and kept under precise condition of temperature 23±2°C and humidity 50-60%. Five rats were housed in each plastic cage measuring 32"x18"x16". Rats were kept throughout the trial on a 12/12h light and dark cycle through nonstop access to rat chow and tap water. The use of animals in this research was in agreement with the National Institute of Health (NIH) guide for the care and usage of Laboratory Animals (Council 2011) and permitted by the Board of Advance Studies and Research University of Karachi.

Dosing

Citrus sinensis

The commonly available variety of Orange (*Citrus sinensis*) family Rutaceae was purchased from local vendors. The fruit was recognized by Plant Conservation Center, Karachi University and receipt sample no C.S 10-10 was deposited to Department of Pharmacognosy, University of Karachi. Fresh juice was squeezed out of the fruit after peeling the fruit, which was used promptly after filtration. *C. sinensis* juice was given orally in three doses according to body weight i.e. 2ml/kg, 5ml/kg and 8ml/kg and was considered as low (LCSD), moderate (MCSD) and high *C. sinensis* dose (HCSD).

Citrus paradisi

The commonly available variety of grapefruit (*Citrus paradisi*) family Rutaceae was also purchased from local vendors. The fruit was recognized by Plant Conservation Center; Karachi University and receipt sample no C.P 09-10 was deposited to Department of Pharmacognosy,

University of Karachi. Fresh juice was squeezed out of the fruit after peeling the fruit, which was used promptly after filtration. *C. paradisi* juice was given orally in three doses according to body weight i.e. 0.1ml/kg, 0.3ml/kg and 0.5ml/kg and was considered as low (LCPD), moderate (MCPD) and high *Citrus paradisi* dose (HCPD).

Combination of C. sinensis and C. paradisi

C. sinensis and *C. paradisi* juices were given in combination by mouth in two doses i.e. 2ml/kg *C. sinensis* juice+0.1ml/kg *C. paradisi* juice and 5ml/kg *C. sinensis* juice+0.3ml/kg *C. paradisi* juice respectively and was abbreviated as SPJ-1 (2+0.1ml/kg) and SPJ-2 (5+0.3ml/kg) respectively.

Induction of inflammation

Wister rats were used to assess anti-inflammatory effect on induced intestinal colitis; Colonic inflammation was induced with trinitrobenzene sulfonic acid (TNBS) as defined by Morris *et al* (1989). Overnight fasted rats were administered 10mg of TNBS dissolved in 0.25 ml of 50% ethanol (v/v) and administered rectally up to 8 cm in the colon through teflon cannula. Rats were held in a head down position for 2-3 minutes after administering TNBS in order to distribute the agent within entire colon. Control animals received phosphate buffer saline (PBS).

Design of experiment

One hundred and ten rats were separated into eleven groups, each containing ten animals. Distribution of animals in different groups was as follows: One group designated as control was given water for injection, with no inflammation, another group designated as inflammation control was given water for injection after induction of inflammation, third group of animals was given standard drug prednisolone after induction of inflammation and eight groups after induction of inflammation were considered treated, three groups were given *C. sinensis*, three groups were given *C. paradisi* and remaining two groups were given combination doses of *C. sinensis* and *C. paradisi*. Administrations of drugs and juices to rats were done for 15 days from the day first of colitis induction to the day before sacrifice of the animals. Entire study was performed under NCCL guideline (Wayne 1998).

Prednisolone was used as standard anti-inflammatory drug after the induction of inflammation and was given in the dose of 0.7mg/kg after diluting in water for injection (Ziesche *et al*. 1998).

Biochemical assay

After completion of the trial, overnight fasted animals were forfeited by decapitation and blood specimens were collected in gel and sodium citrate tubes.

Subsequently colon of the sacrificed animals was detached and positioned on an ice-cold plate and opened longitudinally for histopathological studies. Segments of colon were then weighed and lengths measured under constant load of 2gm. Serum of blood collected in gel tubes was separated by Humax 14K centrifuge at 2000 rpm for 10 min and following parameters were analyzed.

Myeloperoxidase assay

Myeloperoxidase (MPO) level was measured by using the Anti-Myeloperoxidase ELISA (IgG) kit from EUROIMMUN, following manufacturer's manual for instructions. The ELISA kit provides assay for auto-antibodies of the IgG class against MPO in serum or plasma. Photometric measurement of the color intensity was made at a wavelength of 450±2nm. MPO activity is defined as the extent of enzyme degrading 1RU of peroxide per minute at 37◦C and was shown in unit per ml of serum.

Glutathione assay

Glutathione (GSH) level was measured by using the Glutathione ELISA kit from Cusabio Biotech Co., Ltd. The micro titer plates were pre coated with specific GSH. Samples were then transferred to the proper micro titer plate wells with a biotin-conjugated antibody formulation explicit to GSH and incubated after adding Avidin conjugated to Horseradish per oxidase (HRP). Then tetra-methyl-benzidine (TMB) substrate solution was added to each well. The enzyme substrate reaction was aborted by adding sulfuric acid and change in color was estimated spectrophotometrically. Standard curve was prepared by standard plasma and absorbance at 450±2nm was determined. The activity level of GSH in the samples was presented in terms of its percentage with regards to the activity level of standard plasma.

Alkaline phosphate assay

Alkaline phosphate (ALP) was measured on Humalyzer 3000 Human Germany using commercial kit of ALP Human Diagnostic, Germany. 20µl samples were mixed 1000µl Buffer and incubated for 1 minute. Then 250µl substrate p-Nitro phenyl phosphate was added and absorbance was read after 1 minute.

C-reactive protein assay

C-reactive protein (CRP) concentration was measured using commercial kit of CRP Human Diagnostic, Germany as described by Nilsson (1968). All the reagents and samples were allowed to reach room temperature. Then 40 µl samples and one drop of each positive and negative control were placed into distinct circles on the slide test. Then CRP-latex reagent was swirled gently and placed one drop next to the samples, positive control and negative control respectively and mixed gently with separate sticks for 2 minutes then observed under bright light. All parameters were performed as per the guidelines of commercial kits of Human, Germany.

Histopathological examination

Histopathological examination was carried out using procedure described by Diab *et al* (2012). Tissue samples for test and control animals were selected at random.

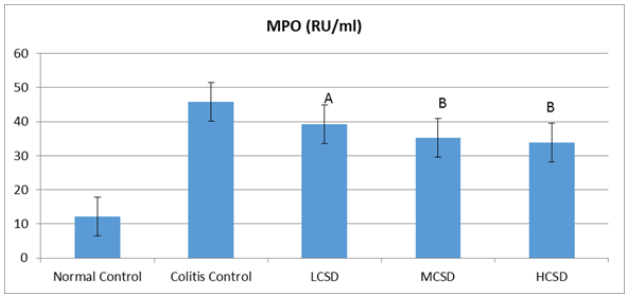
STATISTICAL ANALYSIS

Data entry and analysis was done by SPSS version 17. Data was presented as mean ± S.E.M per 95% sureness interval. ANOVA monitored by post hoc was carried out for comparisons of values with control. Values of $p \leq 0.05$ were considered significant and $p \leq 0.005$ as highly significant

RESULTS

Inflammation profile

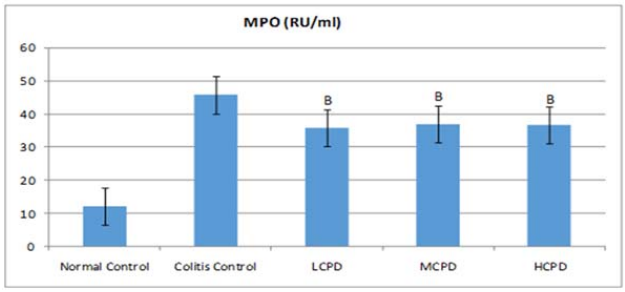
Fig. 1 Illustrates the comparison of MPO levels in normal control, colitis control and three doses of *C. sinensis* treated colitis. There was highly significant reduction in colitis at MCSD and HCSD. However it was significant at LCSD when compared to colitis control group.



Number of animals=10 P≤0.05 (A) P≤0.005 (B)

Fig. 1: Effect of *C. sinensis* in rat colitis as indicated by reduction in MPO levels

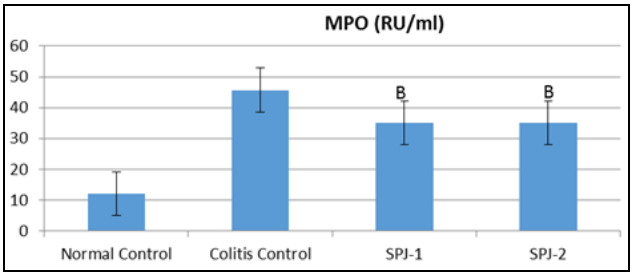
Fig. 2 Illustrates the comparison of MPO levels at normal control, colitis control and three doses of *C. paradisi* treated colitis. There was highly significant reduction in MPO at all three doses of *Citrus paradisi* when compared to colitis control group.



Number of animals=10 P≤0.005 (B)

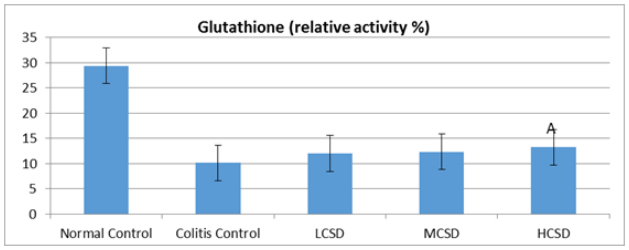
Fig. 2: Effect of *C. paradisi* in rat colitis as indicated by reduction in MPO levels

Fig. 3 shows the comparison of MPO levels in normal control, colitis control and two combination doses of *C. sinensis and C. paradisi* treated colitis. There was highly significant reduction in MPO levels at SPJ-1 and SPJ-2 colitis animal when compared to colitis control.



Number of animals=10 P≤ 0.005(B)

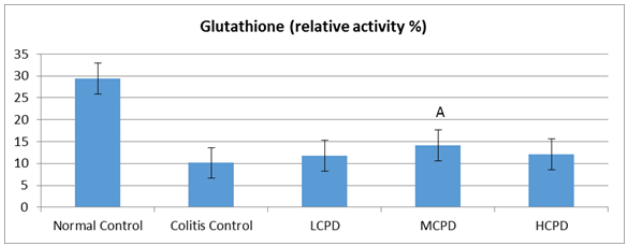
Fig. 3: Effect of combination doses of *C. sinensis* and *C. paradisi* in rat colitis as indicated by reduction in MPO levels



Number of animals=10 P≤0.05(A)

Fig. 4: Effect of *C. sinensis* in rat colitis as indicated by enhanced Glutathione levels

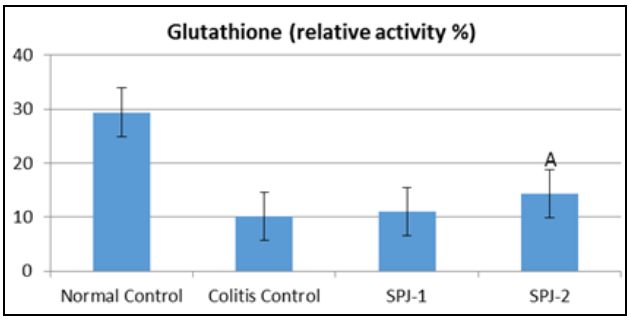
Fig. 4 shows the comparison of GSH levels in normal colitis, colitis control at three doses of *C. sinensis* treated colitis. There was significant increase at HCSD colitis animal when compared to colitis control animals. However there was no significant change observed at LCSD and MCSD colitis animal when compared with colitis control.



Number of animals=10 P ≤ 0.05 (A)

Fig. 5: Effect of *C. paradisi* in rat colitis as indicated by enhanced Glutathione levels

Fig. 5 shows the comparison of GSH levels in normal colitis, colitis control and three doses of *C. paradisi* treated colitis. There was significant increase in GSH levels at MCPD colitis animals when compared to colitis control animals. However there was no significant change observed at LCPD and HCPD colitis animals when compared with colitis control.



Number of animals=10 P ≤ 0.05(A)

Fig. 6: Effect of combination doses of *C. sinensis* and *C. paradisi* in rat colitis as indicated by enhanced Glutathione levels.

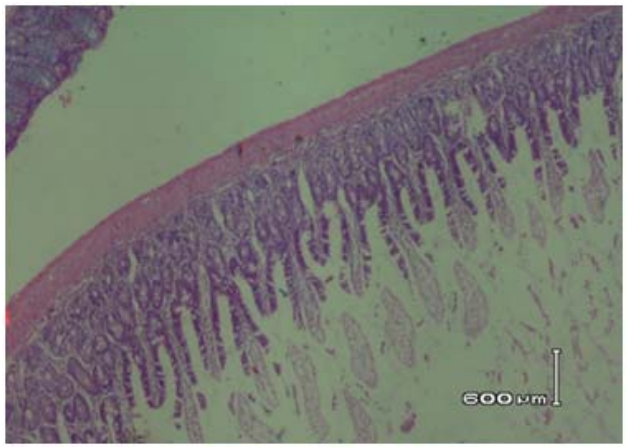


Fig. 7: Intestinal tissues of Colitis control showing damaged cells

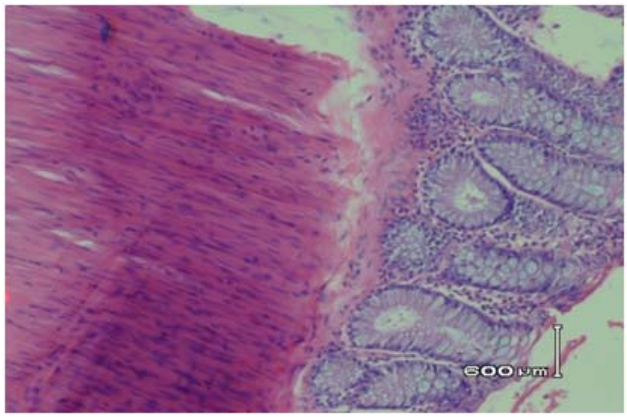


Fig. 8: Intestinal tissues showing normal cells in colitis rats treated with different doses of *C. sinensis*

Fig. 6 shows the comparison of GSH levels in normal, control and colitis treated with two combination doses of *C. sinensis* and *C. paradisi*. There was significant increase at SPJ-2 colitis animals when compared to colitis control animals. However there was no significant change observed at SPJ-1 colitis when compared with colitis control.

Table 1 shows result of *C. sinensis* on ALP and CRP in colitis induced rats and control animals. There was highly significant decrease in ALP and CRP at HCSD in comparison to colitis control. While significant decrease in CRP was observed at MCSD. However there was no significant change at LCSD as compared to colitis control.

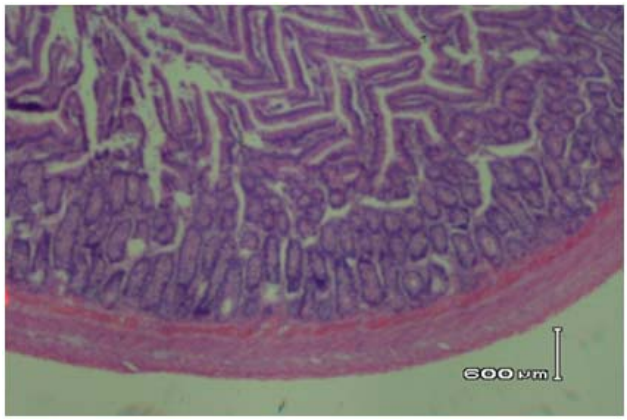


Fig. 9: Intestinal tissues showing normal cells in colitis rats managed by different doses of *C. paradisi.*

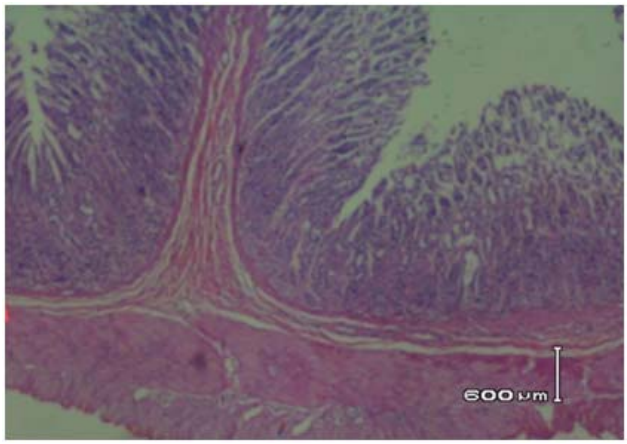


Fig. 10: Intestinal tissues showing normal cells in colitis rats kept on combinations of *C. sinensis* and *C. paradisi.*

Table 2 reveals results of *C. paradisi* on alkaline phosphate level (ALP) and CRP in colitis induced rats and control animals. There was highly significant reduction in ALP level at HCPD and significant decrease in CRP. However there was significant decrease in ALP and CRP level at MCPD. While there was no significant change in the levels of ALP and CRP at LCPD in comparison to colitis control.

Table 3 shows results of two combination doses of *C. sinensis and C. paradisi* on alkaline phosphate level (ALP) and C Reactive Protein (CRP) in colitis induced rats and control animals. There was highly significant decline in ALP at SPJ-2 and significant decrease at SPJ-1 as compared to colitis control. Whereas there was

Table 1: Protective effect of *C. sinensis* in rat colitis

Parameters	Groups/Dose (ml/kg/day)				
	Normal Control	Colitis Control	*c. sinensis* 2	*c. sinensis* 5	*c. sinensis* 8
ALP (U/L)	285.9±26.62	643.6±91.47	452.8±48.53	380.01±22.94	303.0±30.82**
CRP (mg/dl)	0.57±0.04	1.78±0.053	1.72±0.062	1.38±0.167*	1.34±0.15**

Table 2: Protective effect of *C. paradisi* on rat colitis

Parameters	Groups/Dose (ml/kg/day)				
	Normal control	Colitis control	*c. paradisi* 0.1	*c. paradisi* 0.3	*c. paradisi* 0.5
ALP (U/L)	285.9±26.62	643.6±91.47	400.29±31.12	343.6±27.34*	303.57±23.33**
CRP (mg/dl)	0.57±0.04	1.78±0.053	1.40±0.15	1.39±0.16*	1.35±0.17*

Values are mean ± S.E.M
*P ≤0.05 significantly different as associated to control
**P≤0.005 different highly significant from control

Table 3: Effect of combination doses of *C. sinensis and C. paradisi* on rat colitis

Parameters	Groups/Dose (ml/kg/day)				
	Normal control	Colitis control	SPJ 1	SPJ 2	Prednisolone Group
ALP (U/L)	285.9±26.62	643.6±91.47	353.25±27.95*	312.49±17.67**	295.33±26.31**
CRP (mg/dl)	0.57±0.04	1.78±0.053	1.34±0.16*	1.31±0.18*	1.30±0.172*

Values are mean ± S.E.M
*P ≤ 0.05significantly different from control. **p≤ 0.005 different highly significant from control
SPJ 1=2ml/kg *C. sinensis*+0.1ml/kg *C. paradisi*, SPJ 2=5ml/kg *C. sinensis* +0.3ml/kg *C. paradisi*, Prednisolone= 0.7mg/kg

considerable decline in CRP at SPJ-1 and SPJ-2 in comparison to colitis control.

Histopathological profile

Histopathological examination of intestinal tissue of colitis control shows damaged cells (fig. 7). Histopathological examination of intestinal tissue of animals received three doses of *C. sinensis*, *C. paradisi* and two combination doses of these juices did not revealed any microscopic changes in intestinal tissue (figs. 8, 9 and 10).

DISCUSSION

IBD is a chronic inflammatory condition containing two major types; Ulcerative colitis and Crohn's disease. The etiology of IBD is not clear yet. However it is identified that it may be due to association between immune and genetic factors possibly leading to chronic intestinal inflammation categorized by alternating periods of remission and active inflammation (Katz *et al*., 1999).

Chronic inflammation is connected with the production of reactive oxygen species that may contribute to or even initiate an inflammatory reaction. It is also recognized that oxidative stress is a chief factor responsible for the expansion of colorectal cancer in patients of inflammatory bowel disease (Kim and Chang 2014). Kruidenier *et al* (2003) stated that oxidative stress is amplified and antioxidant defense are diminished in colonic mucosa biopsies of patients with IBD. Hence antioxidant compounds may be valuable in restricting injury caused in IBD.

In fact, it has been suggested that the free radical scavenger action may be responsible for the beneficial effects revealed by sulphasalazine and other amino salicylates used in the treatment of human IBD (Ahnfelt-Ronne *et al*., 1990; Grisham 1994). Prior studies have revealed the favorable effects of natural antioxidants in experimental models of rat colitis, including the flavonoids (Galvez 2001b).

Thus in this study TNBS was used to induce inflammation, since these models are useful for reasoning the nature of protective and therapeutic anti-inflammatory response of various agents in IBD.

Marked reduction in histopathological damage observed by *C. sinensis* and *C. paradisi* reveals their protective role against inflammation. It showed significant increase in glutathione (GSH) and highly significant reduction in myeloperoxidase (MPO), which are important biomarkers of inflammation. Reduction in GSH can weaken the cell's defense and may cause cell injury (Micheli *et al*., 1992).

The effect of citrus juices in inflammatory situations may be owing to antioxidant properties of these compounds observed in this study and others (Paya *et al*., 1992). It is also evident by the reinstatement of colonic glutathione

content. Glutathione is crucial in adjusting the redox state of the cell by many mechanisms, together with the scavenging of reactive oxygen species and presence of the enzyme glutathione per oxidase in a reduced state (Sies 1999). Decrease glutathione contents are indicative of oxidant stress, noticed in humans (Morgenstern 2003) and experimental model of colitis (Sanchez De Medina *et al*. 1996; Distasi *et al*., 2004; Camuesco *et al*., 2005).

It has been stated that glutathione supplementation recovers oxidative damage in TNBS colitis (Loguercio *et al* 2003). The enrollment and initiation of leukocytes results in arise in free radical formation that overcomes the tissue's antioxidant protective mechanisms, causing a situation of oxidative stress, which ultimately propagates colonic inflammation (Yamada and Grisham 1994).

A speedy blockade of free radical generation may lower the level of leukocyte penetration into the reddened tissue, thus avoiding colonic tissue to become inflamed (Cestari *et al*. 2011). Additionally, the anti-inflammatory activities may also be related to their inhibitory actions on cyclooxygenase and lipoxygenase pathways (Neichi *et al*., 1983; Loggia *et al*., 1988).

These flavonoids have been described to lessen eicosanoid formation by acting on 5-lipoxygenase and cyclooxygenase rather than by affecting phospholipase A_2 (Paya *et al*., 1992), how ever flavonoids also preventmyelo per oxidase and alkaline phosphatase actions in the intestinal inflammatory procedure. Both enzymes are important biochemical markers of active inflammation in experimental models. MPO is a marker of neutrophil penetration and its activity has been usually measured to perceive intestinal inflammatory process (Yamada *et al*., 1992; Villegas *et al*., 2003).

Declines in myeloperoxidase activity can be understood as an indicator of the anti-inflammatory activity of a given compound (Veljaca *et al*., 1995). Alkaline phosphatase is also a profound biochemical marker of intestinal inflammation (Sanchez De Medina *et al*., 1996; Gonzáles *et al*., 2001) that is augmented due to induction of tissue-nonspecific isoform (Sánchez de Medina *et al*., 2004). Thus, considering the macroscopic and biochemical achievements of the tested compounds, both *C sinensis* and C. *paradisi* showed a preventative effect against the acute intestinal injury encouraged by TNBS in rats, and increase level of MPO and CRP shows plaque instability, coronary heart disease and inflammatory process (Haegens *et al*. 2008; Smith 2010). Reduced level of plasma ALP and CRP may result in anti-inflammatory effect; since there is evidence that for inflammatory response these are important component (Ford *et al*., 2001). These anti-inflammatory effects might be due to the presence of flavonoid contents since flavonoids have strong anti-oxidative and radical scavenging activities.

This effect of flavonoid is due to the regulation of cellular activities of mast cells, lymphocytes, macrophages, neutrophils and modulation of inflammatory mediator enzymes, such as phospholipase A2, arachidonic acid, cyclooxygenase, nitric oxide, nitric oxide synthase and lipoxygenase. Moreover they control expression rate of target gene by modulation of mitogen activated protein kinase (MAPK), protein kinase C (PKC) and reduce DNA binding capacity of factors like NF-κB of transcription and activator protein (Kim *et al*., 2004). On the basis of these results it could be postulated that *C. paradisi* at high dose, *C. sinensis* in dose dependent manner and SPJ-2 exhibit maximum protective role against inflammation.

CONCLUSION

The present study shows that *C sinensis* and *C paradisi* prevented colonic injury caused by TNBS in rats. This effect can be recognized due to the antioxidant and anti-inflammatory properties of these juices, an effect related with a progress in intestinal oxidative stress, thus supporting the upcoming application of these fruits in the management of human IBD. However more investigations are needed to elucidate the molecular mechanisms complicated in these effects.

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