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Evidence of attenuation of intestinal ischemia–reperfusion injury following pre-treatment with methanolic extracts from *Chromolena odorata* in rats

Abstract

Background: *Chromolena odorata* is a tropical species of flowering shrub in the family Asteraceae, leaves of it have been reported to be widely used as herbal remedy for the treatment of various ailments. It is particularly reported to be useful in the healing of wounds.

Methods: We investigated the possibility of amelioration of intestinal ischemia–reperfusion (IR) injury in rats treated with methanolic extract of *C. odorata* (MECO). Wistar albino rats were divided randomly into five groups of six animals each as control, IR-treated, IR + 200 mg/kg MECO, IR + 400 mg/kg MECO, and IR + 200 mg/kg vitamin C. Pre-treatment with MECO or vitamin C was for 7 days.

Results: The contents of hydrogen peroxide (H_2O_2) and malondialdehyde (MDA) were significantly reduced by MECO and vitamin C, while there were significant enhancements of the activities of superoxide dismutase (SOD), glutathione peroxidase (GPX), catalase (CAT), as well as the content of reduced glutathione (GSH) in pre-treated rats compared to IR-treated rats. Glutathione S-transferase (GST) activity was not significantly affected in all the groups. Histopathological examination of small intestinal mucosa revealed significant attenuation of intestinal pathology in animals pre-treated with MECO, while IR injury produced severe villi erosion, necrosis, and inflammatory cell infiltrations.

Conclusions: The present study highlights the antioxidant activities of MECO and its ability to inhibit inflammatory cell infiltration as mechanisms involved in its protection against IR injury in the intestine of rats, an effect that was largely comparable to that of vitamin C.

Keywords: antioxidants, ascorbic acid, *Chromolena odorata*, intestine, ischemia–reperfusion injury

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Introduction

Many surgical procedures involving the gastrointestinal tract often involve or require the occlusion of blood supply to control bleeding and re-establishment of blood flow following the procedure. These operations are often complicated by ischemia–reperfusion (IR) injury that causes destruction to the intestinal tissue and to other close and distant tissues [1]. Cellular damage after reperfusion of previously viable ischemic tissues is described as IR injury. Other clinical-surgical conditions that produce IR injury include interruption of blood flow to the gut as in abdominal aortic aneurysm surgery, cardiopulmonary bypass, strangulated hernias, neonatal necrotizing enterocolitis, and intestinal transplantation [2]. Others include septic and hypovolemic shock [3, 4].

IR injury is very important as it causes high mortality and morbidity [5], via mechanisms that result in systemic inflammation and multiple organ failure [6]. Destruction of epithelial barrier due to IR injury facilitates increased gut permeability with resultant translocation of bacteria and toxins from the gastrointestinal lumen into the systemic circulation [7, 8]. Furthermore, several studies have identified alterations in the absorptive function of the intestine as another major consequence of IR injury [9–11].

Pathophysiological mechanisms involved in the development of IR injury are complex and include multiple mechanisms involving inflammatory cell infiltration, cytokine production, and the production of reactive oxygen species (ROS) [12]. The involvement of ROS in the pathophysiology of IR injury has been well documented [13–15]. The exacerbation of tissue injury in reperfusion appears to be the hallmark of tissue destruction. Ischemia alone causes tissue damage and eventual death, but further injuries can occur while oxygen is reintroduced to the tissue [16].

In ischemic tissues, there is deprivation of oxygen supply (hypoxia) which causes the enzyme xanthine dehydrogenase to be converted to xanthine oxidase, which uses oxygen as substrate rather than nicotinamide adenine dinucleotide. Hypoxia causes a build-up in the levels of hypoxanthine (a degradation product of ATP), as xanthine oxidase is unable to convert hypoxanthine to xanthine. With re-introduction of large amounts of oxygen to the tissues, during reperfusion, xanthine oxidase transfers electrons from the excess hypoxanthine to molecular oxygen, forming large amounts of superoxide radicals [2]. Superoxide radicals can react with hydrogen peroxide in the Haber–Weiss reaction to form the highly reactive hydroxyl radical which promotes the chain of lipid peroxidation and the formation of highly toxic aldehydes such as malondialdehyde (MDA) and 4-hydroxy nonenal (4-HNE).

Treatment of patients undergoing surgery with compounds that can act as antioxidants to scavenge ROS is believed to represent a viable/potential means of alleviating tissue injury. Several efforts in this regard have included the use of various compounds in experimentally induced IR injury. These include allopurinol [17, 18], sucralfate [19, 20], nitroglycerine [21, 22], N-acetylcysteine [23–25], mannitol [26], ellagic acid [27], vitamin E [28, 29], and vitamin C [29, 30]. Vitamin C (ascorbic acid) has shown potential for amelioration of tissue injury following IR [31, 32]. Ascorbic acid, being water-soluble, is believed to wash out free radicals produced during ischemia. Its reducing and chelating properties are also believed to make it potent in scavenging reactive oxidants produced immediately after reperfusion [31].

More recently, substances of botanical origin have gained immense interest among researchers as sources of potent antioxidants to combat tissue injury due to ROS. Against IR injury, *Astralagus membranaceous* [33], *Nigella sativa* [34], and *Trigonella foenum graecum* [35] are examples of research efforts in this regard. Previous attempts in our laboratory have assessed the potential of *Ocimum gratissimum* in intestinal IR injury in rats [36].

Chromolaena odorata, belonging to the family Asteraceae, is a perennial shrub native of South and Central America. It is a serious weed in the humid tropics of South East Asia, Africa, and Pacific Islands. Traditionally, the plant has found wide applications for its medicinal properties. It is applied externally in the treatment of wounds and skin infections. Studies have demonstrated that extracts from the leaves have antioxidant, anti-inflammatory, analgesic, anti-microbial, cyto-protective, and many other medicinally significant properties [37]. Phan et al. [38] also showed that extracts from the leaves of *C. odorata* can protect human dermal fibroblast and epidermal keratinocytes against hydrogen peroxide and hypoxanthine – xanthine oxidase-induced damage. Cyto-protective activities of this plant against gastric ulcers [39] have particularly drawn attention to the potential of this plant in protecting against gastrointestinal mucosal damage. The crude ethanol extracts of *C. odorata* have been found to contain phenolic acids (including protocatechuic acid, p-coumaric acid, ferulic acid, vanilic acid, etc.) and lipophilic flavonoid aglycones such as flavanones, flavonols, flavones, and chalcones [38].

As part of on-going efforts to formulate cost-effective measures for therapies in clinical-surgical situations, we investigated the potential of the methanolic extract of *C. odorata* in alleviating damage due to intestinal IR injury. Vitamin C has been chosen as a standard antioxidant to compare the effects of the extract on IR injury.

Materials and methods

Plant material

Leaves of *C. odorata* (L) (RM King and Robinson) were collected from the University of Ibadan premises and were identified and authenticated by Mr Donatus Esimekhuai of the Department of Botany, University of Ibadan, Nigeria. A voucher specimen was deposited at the herbarium, and the voucher number UIH-22385 was assigned.

Extraction: The leaves were air-dried and then powdered with an electric blender. Three hundred and sixty grams of the dried powdered leaf of *C. odorata* was soaked in 3.5 L of pure methanol in a glass container for 72 h. The solvent now containing the crude extract was collected, filtered, and concentrated using a rotary evaporator at a temperature of 40 °C and pressure of 600 mm Hg for 48 h. The concentrated crude methanolic extract was then weighed to be 35.84 g (a yield of 9.96 %) and was stored at 4 °C throughout the period of the experiment.

Chemicals: Vitamin C (ascorbic acid), epinephrine, 5,5′-dithiobis-2-nitrobenzoic acid, hydrogen peroxide, and thiobarbituric acid were purchased from Sigma Chemical Co. (St. Louis, MO, USA). All other reagents used were of analytical grade and were obtained from British Drug houses.

Animal protocol

Thirty (30) Wistar albino rats (200–250 g) obtained from the Experimental Animal Unit of the Faculty of Veterinary Medicine, University of Ibadan were used in this study. They were housed in a well-ventilated rat house and provided with rat pellets and water *ad libitum*. Animal handling was done according to "Guide for the care and Use of Laboratory animals" prepared by the National Academy of Science and published by the National Institute of Health. The ethic regulations have been followed in accordance with national and institutional guidelines for the protection of animal welfare during experiments. The rats were allowed to acclimatize to animal house conditions for a period of 2 weeks before pre-treatment with the extract and vitamin C. The animals were randomly assigned to five groups of six animals each as follows:

Group A: Control rats

Group B: Rats undergoing IR injury only

Group C: Rats pre-treated with 200 mg/kg MECO followed by IR injury

Group D: Rats pre-treated with 400 mg/kg MECO followed by IR injury

Group E: Rats pre-treated with 200 mg/kg vitamin C followed by IR injury

All pre-treatment was done for 7 days, and the doses of MECO [40] and vitamin C [41] were chosen based on previously published experiments.

Surgical protocol

Animals were fasted for 24 h before the surgical induction of IR injury. They were anesthetized with ketamine (40 mg/kg) and xylazine (5 mg/kg) intramuscularly. A ventral midline laparotomy incision was performed followed by induction of ischemia by clamping of the superior mesenteric artery (SMA) using atraumatic microvascular clips for 30 min. The intestines were returned into the abdomen which was temporarily closed with prolene (Ethicon®). The clamp was removed, and reperfusion was allowed for the next 45 min. After the procedure, the animals were sacrificed and small portions (about 5 cm) of the ileum and jejunum were cut and processed for histopathological examination. The remaining portions of the small intestine were rinsed and homogenized using 50 mM Tris–HCl buffer (pH 7.4) containing 1.15% KCl. The homogenate was subjected to cold centrifugation at 4 °C using a speed of $10{,}000 \times g$ for 15 min. The post-mitochondrial supernatant thus obtained was used for estimation of biochemical parameters.

Biochemical assays

Hydrogen peroxide generation

Hydrogen peroxide generation was assessed by the method of Wolff [42]. This method is based on the ferrous oxidation with xylenol orange with the development of a pale pink color complex generated after incubation for 30 min at room temperature which were read spectrophotometrically at 560 nm.

Malondialdehyde

MDA concentration was measured as an index of lipid peroxidation, according to the method of Varshney and Kale [43]. This method is based on the reaction between 2-thiobarbituric acid (TBA) and MDA which is an end product of lipid peroxide during peroxidation. On heating the mixture of TBA with the tissue homogenates, in acidic pH, the product is a pink complex which absorbs maximally at 532 nm. The MDA level of the sample was estimated from the absorbance using an extinction coefficient of $1.5 \times 10^5\ M^{-1}\ cm^{-1}$ according to the formula by Adam-vizi and Sergi [44].

Reduced glutathione

Reduced glutathione concentration was determined using the method of Jollow et al. [45]. This method is based upon the development of a relatively stable yellow color when 5,5′-dithiobis (2-nitrobenzoic acid), Ellman reagent is added to sulfhydryl compound. The chromophoric product resulting from the reaction of Ellman reagent with the reduced glutathione, 2-nitro-5-thiobenzoic acid possesses a molar absorption at 412 nm which was read at the same wavelength in a spectrophotometer.

Glutathione peroxidase

Glutathione peroxidase (GPX) activity was measured by the method of Rotruck et al. [46], in which hydrogen peroxide was used as substrate.

Glutathione S-transferase

Glutathione S-transferase (GST) was estimated by the method of Habig et al. [47] using 1-chloro-2,4-dinitrobenzene as substrate. The method involves the production of a complex formed from the enzymatic conjugation of reduced glutathione with the aromatic substrate, 1-chloro-2,4 nitrobenzene. The complex formed has a characteristic absorption at 340 nm.

Superoxide dismutase

Superoxide dismutase (SOD) assay was carried out by the method of Misra and Fridovich [48]. This method is based on ability of SOD to inhibit the autoxidation of epinephrine at pH involving superoxide ($O^{2\bullet-}$) radical and hence inhibitable by SOD. Briefly, 100 mg of epinephrine was dissolved in 100 mL distilled water and acidified with 0.5 mL concentrated hydrochloric acid. Thirty microliters of sample was added to 2.5 mL 0.05 M carbonate buffer (pH 10.2) followed by the addition of 300 mL of 0.3 mM adrenaline. The increase in absorbance at 480 nm was monitored every 30 s for 150 s.

Catalase

Catalase (CAT) activity using hydrogen peroxide as substrate was measured by the method of Sinha [49]. This method is based on the fact that dichromate in acetic acid is reduced to chromic acetate when heated in the presence of H_2O_2, with the formation of perchromic acid as an unstable intermediate. The chromic acetate then produced is measured spectrophotometrically at 570–610 nm. The reaction mixture containing CAT is allowed to split H_2O_2 for different periods of time. The reaction was stopped at intervals of 1 min by the addition of dichromate/acetic acid mixture, and the remaining H_2O_2 is determined by measuring chromic acetate spectrophotometrically at 570 nm after heating the reaction mixture.

Histopathology

The cut segments of the ileum and jejunum were cut open longitudinally and fixed in 4 % formalin and embedded in paraffin. They were later sectioned and stained with hematoxylin and eosin. The appearance of the intestinal architecture was graded using the Chiu's score for classification of small intestinal injury [50]. The summary of the features for the grading is provided in Table 1.

Table 1 Histological grading of intestinal lesions based on Chiu et al. [50].

Grade	Histopathology
0	Mucosa without changes
1	Well-constituted villi with no cellular lysis nor inflammatory process, but with formation of the Grunhagen's sub-epithelial space
2	Presence of cellular lysis, formation of Grunhagen's space, and increased spacing among the villi
3	Destruction of the free villosities section, presence of dilated capillaries, and inflamed cells
4	Structural destruction of the villosities, with only traces of some villosities, formed by inflamed cells and necrotic material, with hemorrhage and basal glandular ulceration
5	Destruction of all the mucosa, loss of glandular structure with only the amorphous material laying on the sub-mucosa tissue

Statistical analysis

Statistical analyses were carried out using one-way analysis of variance (ANOVA) to compare the experimental groups followed by the Student's t-test using SPSS (Student version 7.5; SPSS Inc., Surrey, UK), and p-Values <0.05 were considered statistically significant.

Results

Table 2 shows the effect of the methanol extract of *C. odorata* (MECO) on hydrogen peroxide generation in the intestine of rats with IR injury. IR injury did not produce significant alteration ($p<0.05$) in H_2O_2 generation in comparison with the control. However, MECO at 200 mg/kg, 400 mg/kg, and vitamin C (200 mg/kg) produced 10.32 %, 13.51 %, and 14.14 % reductions, respectively, in H_2O_2 generation, although these were not statistically significant. As shown in Figure 1, IR injury produced a 21.61 % elevation in MDA concentration compared to control. However, pre-treatment with MECO at 200 mg/kg and 400 mg/kg caused significant reduction ($p < 0.05$) in MDA levels (48.43 % and 32.58 %, respectively) compared with the IR group. Vitamin C also produced a significant reduction (70.40 %) in MDA levels compared to the IR group.

Figure 2 shows the effects of MECO and vitamin C pre-treatment on reduced glutathione (GSH) levels in rats. IR injury produced significant reduction ($p<0.05$) in GSH levels compared to control. Pre-treatment with doses of MECO tested and vitamin C however prevented the reduction in GSH levels and produced significant increases of

Table 2 Effects of methanol extract of *C. odorata* (MECO) on hydrogen peroxide generation and some antioxidant enzymes in intestinal IR injury in rats.

Parameters	Control (normal saline)	IR only	IR + 200 mg/kg MECO	IR + 400 mg/kg MECO	IR + 200 mg/kg Vit. C
H_2O_2	31.81 ± 4.64	31.00 ± 1.01	28.10 ± 7.34	27.31 ± 4.93	27.16 ± 1.88
CAT	204.89 ± 9.56	254.46 ± 25.03[a]	217.61 ± 33.03	256.33 ± 22.64	248.95 ± 30.26
GPX	147.62 ± 9.11	192.04 ± 21.52[a]	156.82 ± 24.11[b]	179.43 ± 21.13	202.38 ± 27.37[a]
GST	0.012 ± 0.005	0.013 ± 0.0061	0.013 ± 0.006	0.010 ± 0.004	0.008 ± 0.0056

Values are expressed as mean ± standard deviation for hydrogen peroxide (H_2O_2) generation (micromole/min/mg protein); CAT (micromole H_2O_2 consumed/minute/mg protein), GPX (units/mg protein) and SOD (units/mg protein). [a]Values differ significantly ($p<0.05$) from control. [b]Values differ significantly ($p<0.05$) from IR only.

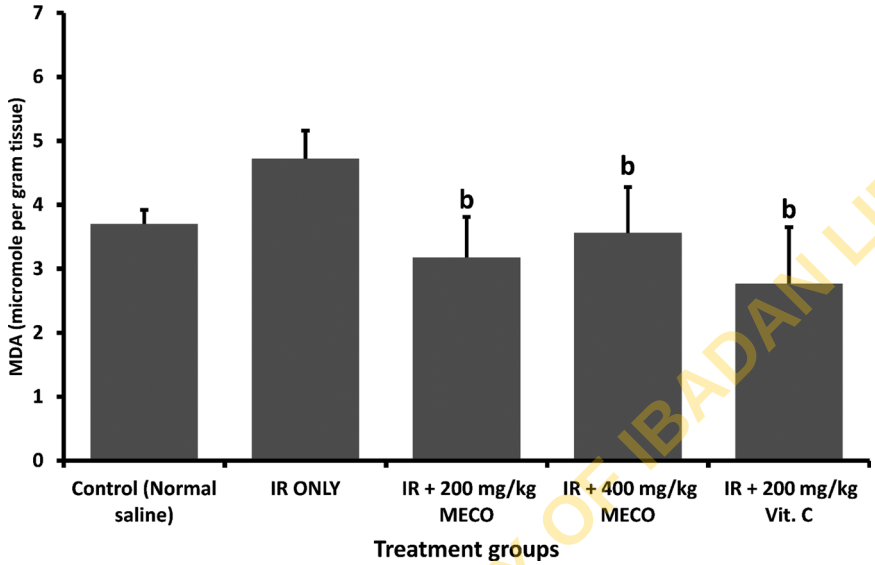

Figure 1 Effect of methanol extract of *C. odorata* and vitamin C on MDA concentration in intestinal IR injury in rats. [a]Values differ significantly from control at $p<0.05$. [b]Values differ significantly from IR only at $p<0.05$.

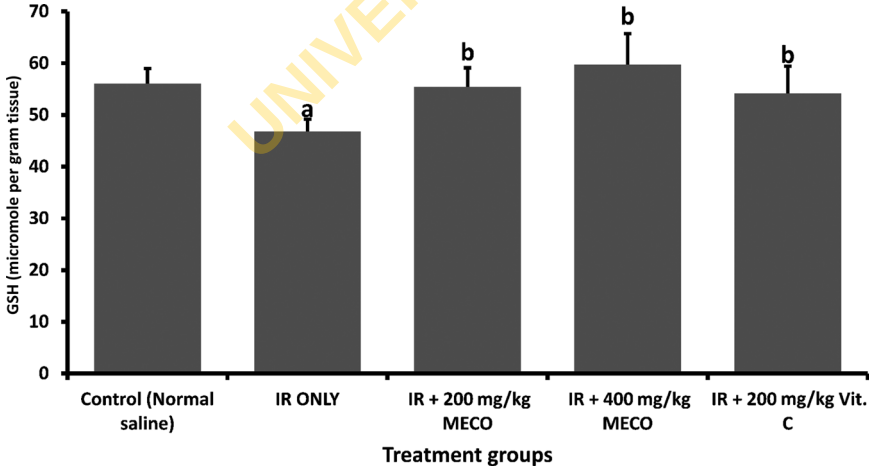

Figure 2 Effect of methanol extract of *C. odorata* and vitamin C on reduced glutathione (GSH) concentration in intestinal IR injury in rats. [a]Values differ significantly from control at $p<0.05$. [b]Values differ significantly from IR only at $p<0.05$.

15.58 %, 21.59 %, and 13.59 % in GSH at 200 mg/kg MECO, 400 mg/kg, and 200 mg/kg vitamin C, respectively, when compared to the IR group. As presented in Table 2, IR injury produced significant induction of GPX activity. Similar induction of GPX activity was also obtained only in rats pre-treated with vitamin C, while the rats pre-treated with the extract did not show appreciable induction of GPX activity compared to the IR group.

CAT activity in rats pre-treated with MECO and vitamin C followed by IR injury is presented in Table 2. There was significant induction ($p<0.05$) in CAT activity in the IR group and the groups pre-treated with either MECO or vitamin C compared to the control. As shown also in Table 2, GST activity was not altered significantly in all the treatment groups.

Figure 3 shows SOD activity in IR injury with rats pre-treated with MECO and vitamin C. IR injury produced a 40.74 % reduction in SOD activity compared to control. MECO at 400 mg/kg and vitamin C prevented the reduction in SOD activity causing significant elevation in SOD activity (35.2 % and 56.45 %, respectively) compared to the IR group.

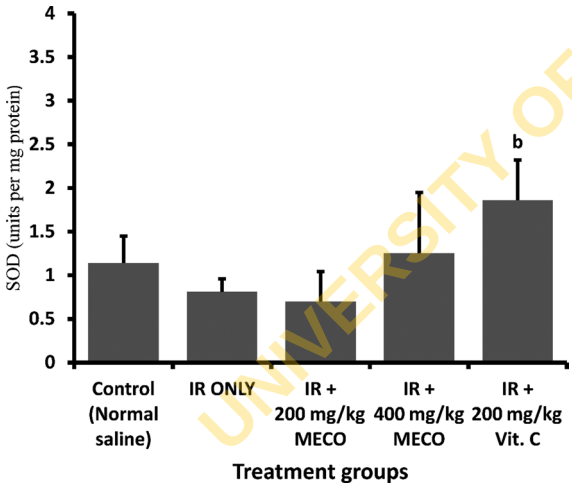

Figure 3 Effect of methanol extract of *C. odorata* and vitamin C on SOD activity in intestinal IR injury in rats.
[a]Values differ significantly from control at $p<0.05$. [b]Values differ significantly from IR only at $p<0.05$.

Microscopy

Representative photomicrographs of the histopathological examination of the intestinal architecture in the different groups of rats are presented in Figures 4 and 5. Rats from the control group had normal architecture of

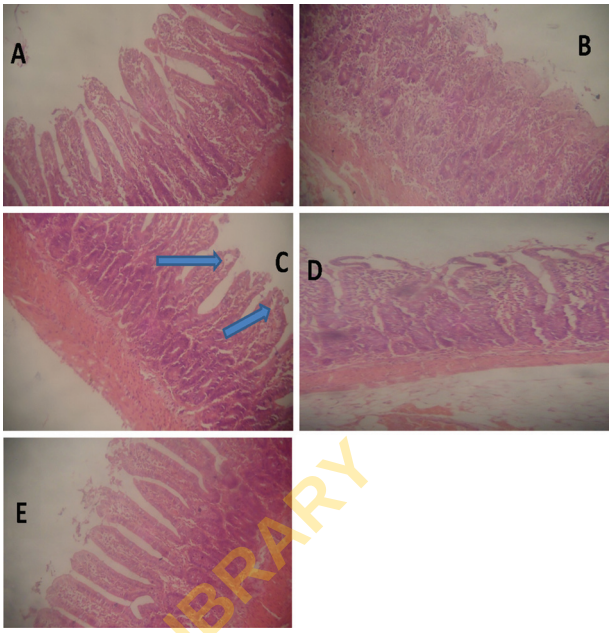

Figure 4 Histological architecture of the ileum in control rats (A), rats undergoing IR injury (B), rats pre-treated with 200 mg/kg MECO followed by IR injury (C), rats pre-treated with 400 mg/kg MECO followed by IR injury (D), and rats pre-treated with 200 mg/kg vitamin C followed by IR injury (E).
Note that the architecture of the intestinal wall was normal (Grade 0) in control rats. In contrast, animals undergoing IR injury alone had severe villi erosion, with severe mucosal and peri-glandular cellular infiltration (Grade 4). Pre-treatment with MECO or vitamin C caused reduction in villi erosion with significant reduction in cellular infiltration (Grades 1 and 2). Grunhagen's space (blue arrows) was evident in Group C rats (magnification × 100).

the epithelium in both the ileum and the jejunum, whereas the rats that underwent ischemia and reperfusion alone had severe villi erosion, with severe mucosal and peri-glandular cellular infiltration of the entire mucosa of the jejunum and ileum. These pathologies were considerably attenuated in the rats pre-treated with MECO in which the higher dose of the extract (400 mg/kg) was more effective in preserving the epithelial architecture. Pre-treatment with vitamin C showed some protection of the intestinal epithelium when compared with the rats undergoing IR alone. However, there were areas of hemorrhage and congestion noticeable in the vitamin C-treated rats.

Discussion

Intestinal IR injury can occur in conditions associated with embolism to the gastrointestinal tract such as

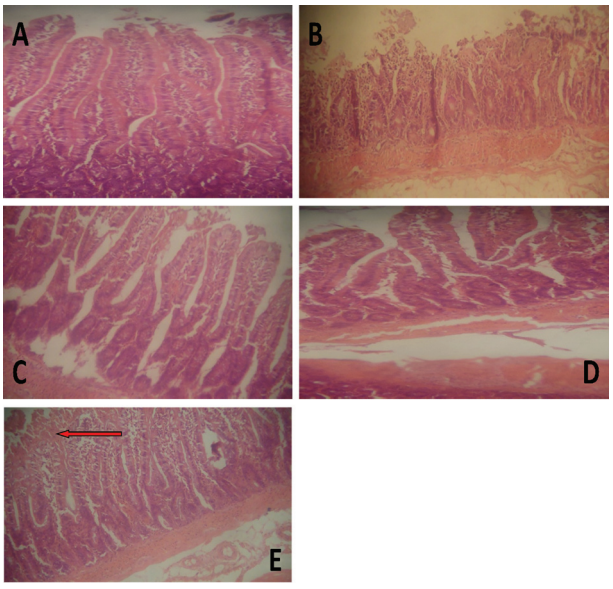

Figure 5 Histological architecture of the jejunum in control rats (A), rats undergoing IR injury (B), rats pre-treated with 200 mg/kg MECO followed by IR injury (C), rats pre-treated with 400 mg/kg MECO followed by IR injury (D), and rats pre-treated with 200 mg/kg vitamin C followed by IR injury (E).
Control rats showed normal architecture of intestinal epithelium (Grade 0). Rats undergoing IR only had severe erosion of the villi and severe cellular infiltration of the entire mucosa (Grade 4). MECO at the two doses and vitamin C prevented the villi erosion and cellular infiltration, although slight hemorrhages and congestion (red arrow) were noticed in the vitamin C group (magnification × 100).

myocardial infarction, artherosclerosis of the aorta, intracardiac thrombus, and so forth. Some other conditions can secondarily predispose to mesenteric ischemia, and they include intestinal adhesions, herniation, volvulus, intussusception, and so forth. The gastrointestinal tract is probably the most sensitive organ to IR injury [12]. Ischemia affects cell metabolism and results in cell death [51]. With reperfusion, cells are prone to generate toxic reactive oxygen metabolites which can cause loss of cell membrane integrity, with release of cellular proteins which activate inflammatory and immune responses, including expression of adhesion molecules, pro-inflammatory mediators, and activation of neutrophils; all of which mediate tissue injury [51, 52].

A major consequence of gastrointestinal IR injury is a compromised mucosal barrier. Increased intestinal permeability can enhance bacterial translocation with production of multiple organ dysfunctions (MODs) [31]. Therefore, ensuring integrity of the intestinal barrier is very important in preventing intestinal pathologies and organ failures. The results of our study showed considerable injury to the ileum and jejunum mucosa after ischemia and reperfusion involving the SMA. However, pre-treatment with *C. odorata* produced significant attenuation of the injury.

We assessed the extent of damage to cellular membranes using MDA as an index of peroxidation of membrane lipids. We found that the MDA content of the tissues was elevated with IR injury. This suggested that the participation of ROS can damage the structure of cell membranes, activating the lipid peroxidation process with production of toxic aldehydes such as MDA [53]. Hydrogen peroxide concentration, assessed in this study, appeared to have played a less significant role in the production of injury. However, its interaction with superoxide radicals to form the more potent hydroxyl radical could have accounted for the increased lipid peroxidation. Togashi et al. [54] found evidence of generation of hydroxyl radical in vivo in the early stage of reperfusion in the heart and liver.

The participation of superoxide radicals in the development of IR injury in this study is reflected in the significant decrease in SOD. SOD is a component of the first line of defense against ROS-mediated injury in tissues. The decline in its activity could indirectly be due to the presence of significant amounts of the superoxide radical.

There were significant reductions in MDA concentrations in animals pre-treated with extracts of *C. odorata* or vitamin C. In addition, there was a progressive enhancement of SOD activity with the doses of MECO and vitamin C, compared to the animals undergoing IR injury alone. This antioxidant activity was found to be higher with vitamin C compared to the extract. This finding supports the reports of earlier studies as vitamin C has been shown to exhibit strong antioxidative action against IR injury [55]. However, dose-dependent protection provided by the extract in some other parameters measured suggests that it may likely offer better protection at higher doses.

Our study showed that *C. odorata* produced a dose-dependent enhancement of GSH concentration, an effect that was higher than that produced by vitamin C. GSH is an endogenous antioxidant which serves as a co-factor for the enzyme GPX. GSH is oxidized to GSSG during metabolism of hydrogen peroxide and lipid peroxides by GPX. GSH can protect against membrane lipid peroxidation by reaction with superoxide radicals. IR injury, in the present study, produced significant reduction in GSH concentration compared to control animals, whereas pre-treatment with *C. odorata* and vitamin C maintained GSH levels at concentrations similar to those of the control animals.

The activity of GPX obtained in this study was significantly elevated following IR injury compared to

control rats. It seems likely that an activation of GPX activity as a means of defense against the oxidants produced during the injury, led to an increased consumption of GSH, with the content of the latter, reducing significantly in IR-treated rats. We found that IR injury after pre-treatment with either MECO or vitamin C also produced elevations in GPX activity, compared to control rats. Interestingly, these increases in GPX activity appeared to correspond to the amount of GSH consumed across the various groups. The reductions in GSH with IR injury would, therefore, appear to be due to increased utilization by GPX, leading to its conversion to GSSG, as well as direct reactions between GSH and free radicals that destroy GSH with formation of products other than GSSG [56]. The ability of MECO to prevent the significant depletion of GSH following IR injury clearly highlights the antioxidant capability of the plant.

CAT activity was significantly elevated in the IR group compared to the control. This could be attributed to some adaptive response to the injury, produced by ischemia and reperfusion. CAT, like SOD, also functions as a component of the first line of antioxidant defense system, and its induction may correlate with attempts by the body to adapt to the injury created. However, elevations in the activities of both CAT and GPX may not be unrelated to the antioxidant activities promoted by both the extracts and vitamin C.

We used the Chiu's model [50] for classifying small intestinal damage to evaluate pathologies to the ileum and jejunum in the different groups. The results of histopathological examination showed that rats in the IR group had severe villi erosion and necrosis, with severe mucosal and peri-glandular cellular infiltration. On the other hand, pre-treatment with *C. odorata* effectively reduced the pathologies observed. In most of the animals, the extract of *C. odorata* was more effective than vitamin C in protecting the intestinal mucosa, as some hemorrhages and congestion of the mucosa were obtained with vitamin C-treated rats. With MECO pretreatment, there was only mild stunting of the villi or isolated areas of mild villi erosion. They were, however, largely devoid of cellular infiltration, hemorrhages, or congestion. The inflammatory response during reperfusion normally triggers recruitment of neutrophils [57, 58], which are believed to play an important role in intestinal IR injury with further potentiation of the cellular injury initiated by ischemia [59]. Therefore, strategies focusing on reducing neutrophil involvement during IR are primary factors affecting recovery and regeneration of injured mucosa during ischemia. *C. odorata* has been reported to have strong anti-inflammatory activities [60], and the results from the histological examination of the intestinal mucosa support this to a large extent.

Conclusions

Our findings provide evidence for protection of intestinal mucosa in IR injury by *C. odorata*, possibly mediated via its antioxidant and anti-inflammatory activities, which are largely comparable to that of vitamin C. Further studies to evaluate the anti-inflammatory potentials of *C. odorata* in IR injury may throw more light into other mechanisms of the protection obtained.

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