Journal of Dietary Supplements

ISSN: 1939-0211 (Print) 1939-022X (Online) Journal homepage: http://www.tandfonline.com/loi/ijds20

Antioxidant Potential of the Methanol Extract of Parquetina nigrescens Mediates Protection Against Intestinal Ischemia-Reperfusion Injury in Rats

Fadeyemi J. Akinrinmade, Akinleye S. Akinrinde, Olubisi O. Soyemi & Ademola A. Oyagbemi

To cite this article: Fadeyemi J. Akinrinmade, Akinleye S. Akinrinde, Olubisi O. Soyemi & Ademola A. Oyagbemi (2015): Antioxidant Potential of the Methanol Extract of Parquetina nigrescens Mediates Protection Against Intestinal Ischemia-Reperfusion Injury in Rats, Journal of Dietary Supplements, DOI: 10.3109/19390211.2015.1103828

To link to this article: http://dx.doi.org/10.3109/19390211.2015.1103828

Journal of Dietary Supplements, Early Online:1–13, 2016

DOI: 10.3109/19390211.2015.1103828

Taylor & Francis
Taylor & Francis Group

ARTICLE

Antioxidant Potential of the Methanol Extract of *Parquetina nigrescens* Mediates Protection Against Intestinal Ischemia-Reperfusion Injury in Rats

Fadeyemi J. Akinrinmade[1], Akinleye S. Akinrinde[2], Olubisi O. Soyemi[1], & Ademola A. Oyagbemi[2]

[1]*Department of Veterinary Surgery and Reproduction, Faculty of Veterinary Medicine, University of Ibadan, Ibadan, Nigeria,* [2]*Department of Veterinary Physiology, Biochemistry and Pharmacology, Faculty of Veterinary Medicine, University of Ibadan, Ibadan, Nigeria*

ABSTRACT. *Parquetina nigrescens* is a medicinal herb with recognized antioxidant properties and potential to alleviate conditions associated with oxidative stress, including gastric ulcers. We investigated the protective potential of methanol extract of *Parquetina nigrescens* (MEPN) against ischemia-reperfusion injury in the intestine of rats. Thirty (30) male Wistar albino rats were randomly assigned into five groups with Group I made up of control rats and Group II consisting of rats experimentally subjected to ischemia and reperfusion (IR) by clamping of the superior mesenteric artery (SMA) for 30 minutes and 45 minutes, respectively. Groups III and IV rats also had IR, but were initially pre-treated with MEPN at 500 mg/kg and 1000 mg/kg respectively, for seven days. Rats in Group V were also pre-treated with Vitamin C, for seven days, before induction of IR. The results showed marked reduction in intestinal epithelial lesions in groups treated with MEPN, compared to the IR group which had severe villi erosion, inflammatory cell infiltration and hemorrhages. There were significant increases in Malondialdehyde (MDA) and significant reductions in reduced glutathione (GSH) and Glutathione S-transferase (GST) activity with IR injury, while pre-treatment with either MEPN or Vitamin C prevented these effects. Increases in Glutathione peroxidase (GPX), Catalase (CAT) and Superoxide dismutase (SOD) with IR provided evidence for adaptive responses to oxidative injury during IR and preservation of enzyme activity by MEPN and Vitamin C. Taken together, *Parquetina nigrescens* provided considerable alleviation of intestinal injury produced by IR, at values much as effective as that offered by Vitamin C.

KEYWORDS. antioxidant, intestine, Ischemia-reperfusion injury, *Parquetina nigrescens*, Vitamin C

Address correspondence to: Dr. Akinleye S. Akinrinde, Department of Veterinary Physiology, Biochemistry and Pharmacology, Faculty of Veterinary Medicine, University of Ibadan, Ibadan, Nigeria. (Email: as.akinrinde@mail.ui.edu.ng)
Color versions of one or more of the figures in the article can be found online at www.tandfonline.com/ijds.

INTRODUCTION

Ischemia-reperfusion (IR) injury is important in many clinical and surgical procedures, especially those involving the gastrointestinal tract. In fact, the small intestine is believed to be the most sensitive tissue to injury induced by IR (Mojzis et al., 2001; Yamamoto et al., 2001). IR injury to the intestinal epithelium may occur in conditions such as acute mesenteric ischemia, intestinal obstruction, volvulus etc, and the injury may produce impairment in mucosal integrity, leading to decrease in absorption of nutrients and bacterial translocation into the blood stream and other organs (Yamamoto et al., 2001). Strangulating lesions such as colic in horses and the obstructive lesions of the intestines may produce IR injury (Wong, Moore, and Brockus, 2012). Although traditional approaches, such as exploratory celiotomy, may be used to relieve strangulation or obstruction of the intestines, experimental information regarding the underlying pathophysiologic mechanisms contributing to intestinal injury, even after surgery, will go a long way to offer additional therapeutic strategies for animals experiencing intestinal ischemia and reperfusion injury.

Reactive oxygen metabolites formed during ischemia are believed to be important mediators of injury to tissues. Paradoxically, their levels increase profoundly during restoration of blood supply to ischemic tissues. The hypoxia that accompanies a period of ischemia causes an irreversible conversion of xanthine dehydrogenase to xanthine oxidase. Simultaneously, much of the cell's adenosine triphosphate (ATP) is converted to hypoxanthine which serves as a good source of electrons for xanthine oxidase. During reperfusion, when oxygen is supplied in large amounts to the tissues earlier deprived of oxygen, the electrons from xanthine oxidase are transferred to molecular oxygen, forming large quantities of superoxide radicals (Collard & Gelman, 2001). Thus, far greater amounts of oxidants are produced after restoration of blood supply to ischemic tissues. Xanthine dehydrogenase is known to be widely distributed throughout the body, with particularly high concentrations in the intestine and liver (Nordstrom, Seeman, & Hasselgren, 1985), making these tissues highly susceptible to damage by IR.

Due to the great involvement of reactive oxygen species in the pathophysiology of IR injury, antioxidants have been employed by several researchers in an attempt to alleviate the injury produced by IR. Well known antioxidants such as Vitamin C have proved useful in offering protection against post-ischemic and post-reperfusion injury in the intestinal tissues of rats (Nakamura et al., 1997; Higa et al., 2007). The mechanisms involved in the protection involve the scavenging of free radicals and/or the reduction of lipid peroxidation, including the maintenance of glutathione levels (Nakamura et al., 1997). It, therefore, suggests that rich natural sources of antioxidants would prove useful in conditions producing IR injury.

Recently, phenolics from plants have proved to be more potent antioxidants than Vitamins C, E and even carotenoids (Rice-Evans et al., 1995, 1996). It is reasonable to suggest that an integrated total antioxidant power of a complex sample could be more meaningful to evaluate the health benefits of natural compounds because of the cooperative action of antioxidants (Dai & Mumper, 2010). Extracts from plants represent such mixture of compounds that have antioxidant properties including flavonoids, tannins, etc.

We hypothesized that the extract of *Parquetina nigrescens* with its high content of flavonoids, saponins, glycosides, cardiac glycosides, tannins, anthraquinones, phlobatannins and oils and its reported antioxidative properties (Ayoola et al., 2011), should offer protection in clinical situations involving IR injury. Surgical procedures could especially benefit when these antioxidant properties of such extract could be utilized as part of the array of premedication regimen for patients undergoing elective surgery. Therefore, the aim of our study was to investigate the protective role of methanol extract of *Parquetina nigrescens* in intestinal ischemia-reperfusion injury in rats.

MATERIALS AND METHODS

Chemicals and Reagents

Epinephrine, glutathione, 5, 5'-dithiobis-2-nitrobenzoic acid, hydrogen peroxide, thiobarbituric acid, and trichloroacetic acid, were purchased from Sigma Chemical (St. Louis, MO). All other reagents used were of analytical grade and were obtained from British Drug houses.

Collection and Identification of Plant Material

Leaves of *Parquetina nigrescens* were collected from the University of Ibadan campus and were identified and authenticated at the Department of Botany, University of Ibadan, Nigeria. A voucher specimen was deposited at the herbarium and the voucher number UIH-22384 was assigned.

Extraction

Three hundred and eighty grams of the dried, powdered leaves of *Parquetina nigrescens* were soaked in 3.5 litres of pure methanol inside a glass container for 72 hours. The solvent now containing the crude extract was collected, filtered and concentrated using a Rotary Evaporator under a temperature of 40°C. The crude extract was further concentrated using a vacuum oven at a temperature of 40°C and pressure of 600 mmHg for 48 hours. The concentrated crude methanolic extract was then collected and weighed to be 25.90g (a yield of 6.81%) and stored at 4°C throughout the period of the experiment.

Animals and Surgical Procedure

Thirty (30) Wistar rats, weighing 200—250g were used in this study. They were randomly divided into five groups of six animals each. 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 Institutes of Health (NIH). The rats were allowed to acclimatize to animal house conditions for a period of two weeks before pre-treatment with either the extract or Vitamin C. All animals were served normal pelleted diet and water, *ad libitum* throughout the period of the study. Rats in group 1 were the normal (negative) control animals. Group 2 rats (IR only) had ischemia induced in the intestinal tissues by clamping the Superior mesenteric artery for 30 minutes, followed by reperfusion by removal of the clamp

for 45 minutes. Rats belonging to groups 3 and 4 were initially pre-treated with methanol extract of *Parquetina nigrescens* (MEPN) for seven days, before induction of IR, while group 5 animals were pre-treated with Vitamin C for seven days prior to induction of IR. The dosages of *Parquetina nigrescens* (500 mg/kg and 1000 mg/kg) and Vitamin C (200 mg/kg) were chosen according to previous works by Aderibigbe et al. (2010) and Owu et al. (2012), respectively.

For the induction of IR, the rats were anesthetized with Ketamine (40 mg/kg) and Xylazine (5 mg/kg) intramuscularly. The surgical procedure involved a ventral midline laparotomy incision following which the intestinal tissues were dissected out. The Superior mesenteric artery was located and was carefully clamped with an atraumatic microvascular clamp for 30 minutes. Blood flow was re-established for 45 minutes after the period of ischemia. The animals were sacrificed at the end of the experiment and the small intestine from the duodenum to the ileum was harvested. Small portions of the ileum and jejunum were cut for histopathological examination while the remaining tissues were processed for biochemical assays.

Biochemical assays

The intestines were opened along their whole length, washed in ice-cold saline and blotted dry. Twenty-five percent tissue homogenates were prepared 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 xg for 15 minutes. The supernatant thus obtained was used for biochemical assays.

Protein concentration was measured using the Biuret method of Gornal et al. (1949). Lipid peroxidation was determined using Malondialdehyde (MDA) as the biomarker, according to the method described by Farombi et al. (2000). Hydrogen peroxide generation was assessed by the method of Wolff (1994). Reduced glutathione (GSH) was measured using the method of Jollow et al. (1974) based on reaction of sulfhydryl groups of glutathione with 5,5′-dinitro bis (2-nitrobenzoic acid) (DTNB), after the samples have been deproteinized with equal volumes of sulfosalicylic acid. GSH concentration was quantified spectrophotometrically at 412 nm.

Superoxide dismutase (SOD) assay was carried out by the method of Misra and Fridovich (1972), with slight modification in our laboratory. Briefly, 100 mg of epinephrine was dissolved in 100 mL distilled water and acidified with 0.5 mL concentrated hydrochloric acid. Thirty microliters of the intestinal sample were 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. Glutathione S-transferase (GST) activity was assayed by the method of Habig et al. (1974) with 1-chloro-2, 4-dinitrobenzene as substrate. Glutathione peroxidase (GPX) activity was measured according to the method of Rotruck et al. (1973), while Catalase (CAT) activity was measured by the method of Sinha (1971) using hydrogen per-oxide as substrate.

HISTOPATHOLOGY

Small portions of the ileum and jejunum were fixed in 10% formalin, embedded in paraffin wax, and sections of 5–6 mm in thickness were made and thereafter stained

with hematoxylin and eosin for histopathological examination. The sections were examined with light microscopy.

Statistical Analyses

Values have been expressed as mean ± standard deviation. 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). Values < 0.05 were considered statistically significant.

RESULTS

Table 1 presents the values obtained for hydrogen peroxide generation and the activities of some antioxidant enzymes in animals pre-treated with methanol extract of *Parquetina nigrescens* and undergoing Ischemia-reperfusion. Hydrogen peroxide levels were not significantly altered in all the groups in this study. However, the activities of GPX and Catalase were significantly increased ($p < 0.05$) in the groups that had ischemia-reperfusion compared to the control group. This elevation in GPX and CAT activities was highest in the group pre-treated with Vitamin C. SOD activity was also significantly increased in the groups that had IR compared to the control group. However, the induction of SOD activity was higher in the groups pre-treated with MEPN or Vitamin C, compared to the IR group.

From Figure 1, the MDA level was significantly increased in the groups that had IR compared to control. However, the increase was alleviated with pre-treatment with MEPN at 3.26% and 23.72%, respectively, for the 500 and 1000 mg/kg doses, compared to the IR group. Vitamin C produced a 3.78% reduction in MDA compared to the IR group.

As shown in Figures 2 and 3, IR caused a significant reduction ($p < 0.05$) in GSH concentration and GST activity when compared with control values. However, pre-treatment with either MEPN or Vitamin C preserved the levels of GSH with GSH concentrations maintained at or even above control levels. The highest effect of maintenance of GSH level was obtained with the 1000 mg/kg dose of the extract.

TABLE 1. Alterations in Hydrogen peroxide generation and activities of some antioxidant enzymes in the small intestine of rats after ischemia-reperfusion injury

	Group A (control) Normal saline	Group B IR only	Group C IR + 500 mg/kg MEPN	Group D IR + 1000 mg/kg MEPN	Group E IR + 200 mg/kg Vit. C
H_2O_2	36.50 ± 1.06	34.35 ± 2.11	35.33 ± 3.23	36.38 ± 4.16	35.75 ± 3.88
GPX	80.81 ± 10.16	131.30 ± 6.70^{a}	$125.05 \pm 14.88^{a,b}$	$122.21 \pm 12.10^{a,b}$	$143.07 \pm 33.19^{a,b}$
CAT	121.66 ± 12.71	220.68 ± 15.80^{a}	$246.21 \pm 19.67^{a,b}$	233.13 ± 18.60^{a}	$254.80 \pm 17.90^{a,b}$
SOD	0.54 ± 0.06	0.88 ± 0.07^{a}	0.99 ± 0.19^{a}	0.97 ± 0.18^{a}	0.97 ± 0.15^{a}

Values are presented as mean±standard deviation; [a] indicates significant differences at $P < 0.05$ as compared to control; [b] indicates significant differences as compared with IR only. H_2O_2, Hydrogen peroxide (micromole per minute per mg protein); GPX, Glutathione peroxidase (units per mg protein); CAT, Catalase (micromole H_2O_2 consumed per minute per mg protein); SOD, Superoxide dismutase (units per mg protein).

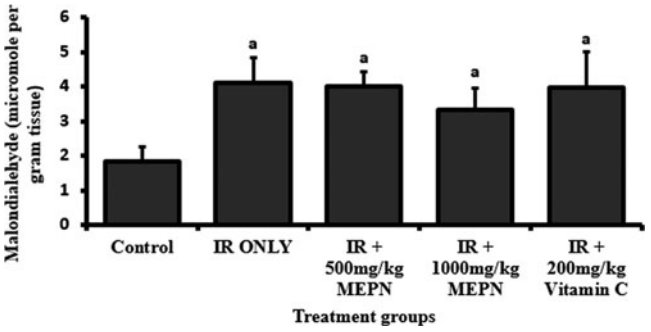

FIGURE 1. Effect of Methanol extract of *Parquetina nigrescens* (MEPN) on Intestinal Malondialdehyde (MDA) concentration following Ischemia-reperfusion injury. [a] indicates significant difference ($p < 0.05$) as compared to control; [b] indicates significant difference ($p < 0.05$) as compared to IR only.

MICROSCOPY

Figures 4 (a, b, c.) show photomicrographs representing the histological architecture of the ileum and jejunum from the rats in the different groups. By light microscopy, it was observed that the normal control rats had the normal architecture of both the ileum and jejunum, with no visible lesions observed. However, considerable pathologies were observed in the rats that had only ischemia-reperfusion. IR produced severe villi erosion with inflammatory cell infiltration and debris, although the glandular area in the submucosal region appears largely intact. Pre-treatment of rats with MEPN prior to induction of IR caused only moderate mucosal congestion and slight epithelial erosion in the ileum at 500 mg/kg MEPN, whereas, pre-treatment with 1000 mg/kg MEPN showed preservation of the epithelium with no visible lesions. Vitamin C pre-treatment produced only mild preservation of IR injury when compared to the animals pre-treated with the extract of

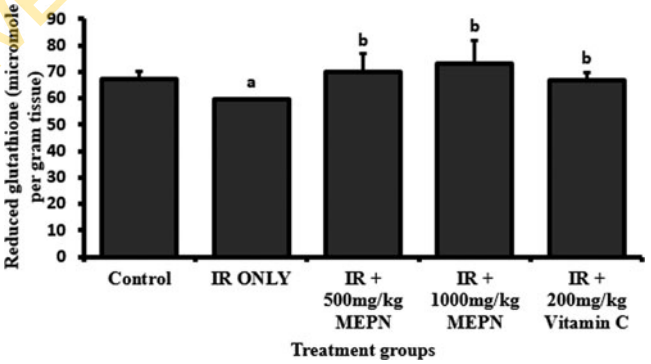

FIGURE 2. Effect of Methanol extract of *Parquetina nigrescens* (MEPN) on Intestinal Reduced glutathione (GSH) concentration following Ischemia-reperfusion injury. [a]indicates significant difference ($p < 0.05$) as compared to control; [b]indicates significant difference ($p < 0.05$) as compared to IR only.

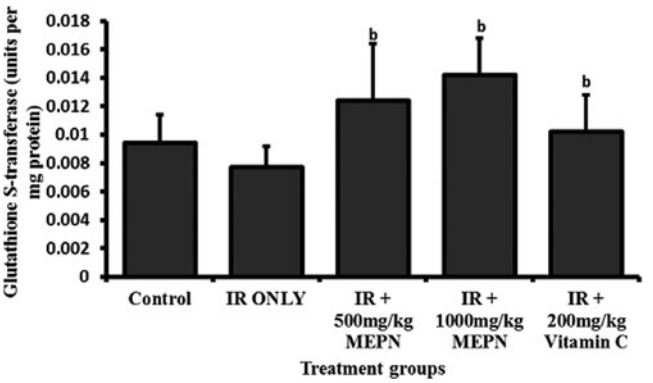

FIGURE 3. Effect of Methanol extract of *Parquetina nigrescens* (MEPN) on Intestinal Glutathione S-transferase (GST) activity following Ischemia-reperfusion injury. [a]indicates significant difference ($p < 0.05$) as compared to control; [b]indicates significant difference ($p < 0.05$) as compared to IR only.

Parquetina nigrescens, as there were areas of mild necrosis of the villi tips, mild cellular infiltration and mucosal debris.

DISCUSSION

One of the main consequences of temporary vascular occlusion is ischemia-reperfusion (IR) injury (Cornet et al., 2009). When this occurs in splanchnic tissues, the aorta or its branches are usually involved and the intestines are often the most sensitive tissues to the injury (de Arruda et al., 2006; Zanoni et al. 2009).

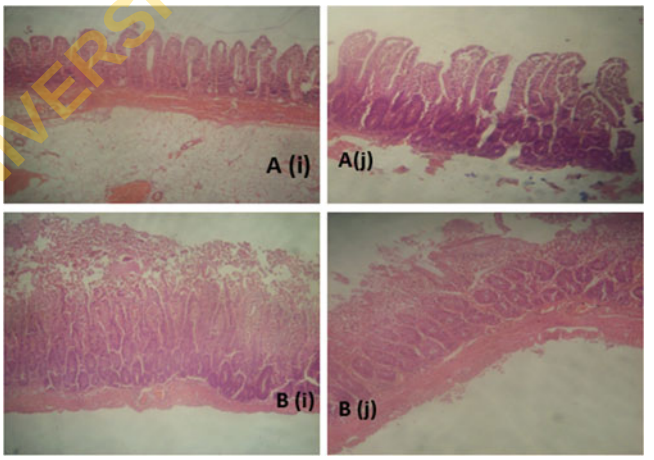

FIGURE 4a. Histological architecture of ileum (i) and Jejunum (j) in normal control rats (A) and rats undergoing ischemia-reperfusion injury (B). Control rats showed no visible lesions in the epithelium of both ileum and jejunum. Ileal and jejunal epithelia in rats undergoing IR injury showed severe villi erosion with inflammatory cell infiltration and debris, although the glandular area in the sub-mucosal region appears largely intact.

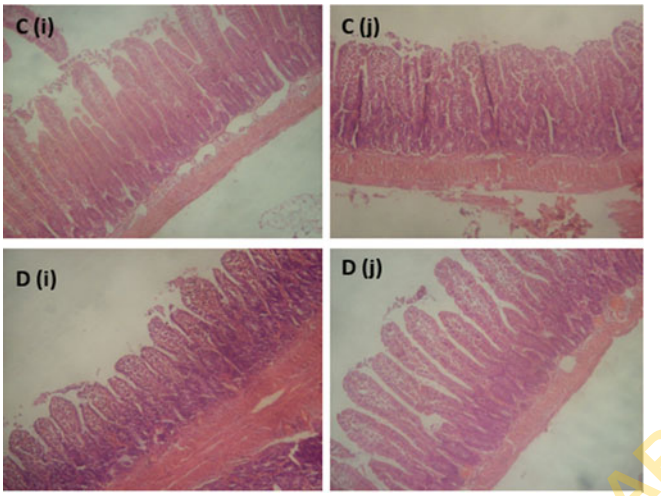

FIGURE 4b. Histological architecture of ileum (i) and Jejunum (j) in rats pre-treated with 500 mg/kg MEPN (C) and rats pre-treated with 1000 mg/kg MEPN (D). Slides show considerable protection of mucosal integrity. Moderate mucosal congestion and slight epithelial erosion were noticed in the ileum at 500 mg/kg MEPN, whereas, pre-treatment with 100 mg/kg showed preservation of the epithelium with no visible lesions.

Although the mechanisms involved in the pathogenesis of IR injury have not been fully elucidated, oxidative stress mediators such as reactive oxygen species (ROS), polymorphonuclear neutrophils (PMNs) and nitric oxide (NO) are believed to be very important. In this regard, a variety of antioxidant compounds have been investigated for their protective benefits in experimental conditions involving ischemia-reperfusion injury (Nakamura et al., 1997; Gunel et al., 1998; Köhler, DeLucca, & Snraglia, 2011). We hypothesized that the array of antioxidant compounds present in plant extract could offer value in preventing oxidative stress-mediated injury in intestinal ischemia-reperfusion. Our experimental model of investigating ischemia-reperfusion injury in the intestine of rats, involving the superior mesenteric artery (SMA) has been well standardized, as the period of ischemia and reperfusion can

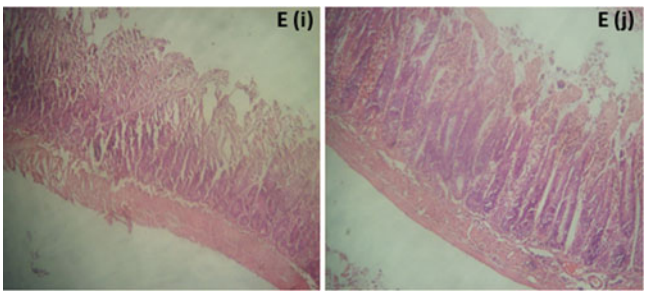

FIGURE 4c. Histological architecture of ileum (i) and Jejunum (j) in rats pre-treated with 200 mg/kg Vitamin C (E). The ileum and Jejunum epithelium had only mild protection of the epithelium as there were areas of mild villi necrosis with inflammatory cell infiltration and some mucosal debris.

be controlled and the method can be applied equally to all experimental groups (Higa et al., 2007; Köhler, DeLucca, & Snraglia, 2011).

In this study, although hydrogen peroxide (H_2O_2) levels were not significantly affected in all the groups, the concentration of Malondialdehyde (MDA) was significantly increased in all the groups of rats that had IR, compared to the control, although the rats that had IR only had the most elevated levels of MDA. These findings point to a significant increase in lipid peroxidation within the period of induction of ischemia and reperfusion. Lipid peroxidation is an important consequence of free radical generation leading to the formation of toxic aldehydes such as MDA (Chauhan, Ojha, & Mahmood, 2011). The extract from *Parquetina nigrescens* caused reductions in the levels of MDA compared to those that had IR only, although the values were still higher than those of normal control animals. Increased MDA despite unaltered H_2O_2 levels suggests that other reactive oxygen species and/or free radicals, including superoxide radicals may be involved in the oxidative injury.

In response to the acute IR injury in this study, the activities of SOD, CAT and GPX were significantly elevated, compared to control. SOD is an antioxidant enzyme that can be found in the cytosol, mitochondria and extracellularly (Nozik-Grayck, Suliman, & Piantadosi, 2005). It catalyses the conversion of excess superoxide anions and converts them to H_2O_2 (Fridovich, 1989). CAT and GPX then scavenge H_2O_2 and convert it to water (Shull et al., 1991; Mates et al., 1999). GPX reduces lipid and non-lipid hydroperoxides as well as hydrogen peroxide via a GSH-dependent mechanism (Michiels & Remacle, 1988). Elevation in GPX and/or CAT activity could be due to an increased demand for these enzymes when levels of hydrogen peroxide or lipid peroxides are increased (Kolodziejczyk, Siemieniuk, & Skrzydlewska, 2005). The increase in the activities of these free radical detoxifying enzymes, GPX and CAT, in the intestines of IR-treated rats observed in the present study may also be an adaptive mechanism by the cells to detoxify the reactive species in order to minimize tissue damage (Kolli, Abraham, & Isaac, 2007).

In our study, it seems likely that the elevated activities of CAT and GPX were due to increased levels of lipid peroxides as there was a significant increase in lipid peroxidation, indicated by elevated levels of MDA. Significant increases in GPX and CAT activity in the animals pre-treated with either the extract of *Parquetina nigrescens* or Vitamin C, when compared with control animals, could be attributed to a combined effect of the stimulation of their activities due to increased levels of peroxides and the preservation of antioxidant activity by the components of the extract and Vitamin C (Mates, Perez-Gomez, & Decastro, 1999). In addition, the levels of endogenous antioxidants may also be up -regulated by increasing expression of the genes encoding the antioxidant enzymes (Semiz & Sen, 2007). Thus, initial pre-treatment with the extract could prove beneficial in this regard. Significant increase in SOD activity during IR also points to an increase in demand for the enzyme in the acute process of IR injury (Mates, Perez-Gomez, & Decastro, 1999). Although, superoxide radical generation was not assessed in this study, we suggest that the elevated levels of SOD could be due to increased levels of superoxide radicals.

GSH, the main component of the antioxidant defense system, is an effective free radical scavenger that participates in important metabolic functions such as

maintenance of protein sulfhydryl groups in the reduced state; enzymatic reactions catalysed by Glutathione S-transferase, Glutathione Reductase, and Glutathione peroxidase; the transport of amino acids; and protein and DNA synthesis (Masella et al., 1999). There was significant reduction in GSH concentrations in rats with IR only, compared to controls. However, rats that were pre-treated with either extract or Vitamin C showed significant preservation of GSH concentrations at levels similar to, or higher than control values.

The decrease observed in GSH levels may be the result of an unbalanced turnover involving increased consumption of GSH in detoxifying peroxides, a reduction in gamma-glutamylcisteinyl synthetase activity, and a lack of availability of cysteine, glycine, and methionine, the limiting amino acids for GSH synthesis (Sido et al., 1998; Nieto et al., 2000). Excess lipid peroxides can reduce the ratio of reduced glutathione concentration to oxidized glutathione (GSSG) and reduce the GSH concentration in cells. The products of lipid peroxidation that react with GSH and at a high intensity of oxidative stress, may lead to ATP consumption and glutathione depletion in cells which can cause damage to cells (Sies & Cadenas, 1985).

Interestingly, the activity of Glutathione S-transferase (GST) exhibited similar changes as that of GSH concentration. There was a significant drop in GST activity following induction of IR, while pre-treatment with *Parquetina nigrescens* or Vitamin C preserved GST at levels similar to, or even greater than that of control rats. GST catalyses the conjugation of GSH with the end products of oxidation and ROS. As such, GST can remove free radicals and its levels can reflect the antioxidant capacity of tissues (Chauhan, Ojha, & Mahmood, 2011). Thus, the observed decrease in GST activity with IR alone may be due to oxidative stress induced during the acute IR injury.

Histologically, it was observed that ischemia and reperfusion of the intestine without *Parquetina nigrescens* or Vitamin C pre-treatment was associated with severe villi erosion with inflammatory cell infiltration and debris. In contrast, rats that were pre-treated with either extract or Vitamin C showed protection of the intestinal architecture, which was more obvious with the extract. The majority of animals pre-treated with *Parquetina nigrescens* showed only mild lesions, which were much reduced at the higher dose of the extract. However, mild to severe villous necrosis was still observed in the rats pre-treated with Vitamin C. Previous histomorphometric assessment of intestinal architecture after IR injury by Higa et al. (2007), had demonstrated increased villous necrosis and hemorrhagic infarcts. In that study, Vitamin C pre-treatment one hour prior to the induction of IR was reported to have reduced the extent of villus necrosis, compared to those animals that were not pre-treated with it. However, in our study, although pre-treatment was carried out for seven days, the longer length of time between the last administration of Vitamin C and the induction of IR (about 24 hours) could have resulted in the lesser protection observed. It is reasonable to suggest that components of the extract may be more persistent than Vitamin C at the mucosal surface where they act to scavenge reactive oxygen species, and this may have resulted in the better mucosal protection obtained with the extract. Vitamin C, being water-soluble, could easily be washed away from the mucosal surface and its benefit in muco-protection may only be realized within a short time after its administration.

In conclusion, the results of our study showed that the methanol extract of *Parquetina nigrescens* possessed considerable potential to alleviate IR injury in the intestines of rats. Our biochemical assays revealed that the intestinal mucosa possessed the ability to stimulate its antioxidant defenses as means of protection against ROS insults generated in the acute IR injury. An important finding in this study is that *Parquetina nigrescens* offered greater protection than Vitamin C against IR, as indicated by histopathological evaluation and some biochemical parameters. *Parquetina nigrescens*, therefore, showed merit for further investigation of its potential in alleviating oxidative-stress mediated injury as well as other possible mechanisms of the protection it could provide intestinal mucosal injury.

Declaration of Interest: The authors report no conflicts of interest.

ABOUT THE AUTHORS

Fadeyemi J. Akinrinmade, Department of Veterinary Surgery and Reproduction, Faculty of Veterinary Medicine, University of Ibadan, Ibadan, Nigeria, **Akinleye S. Akinrinde**, Department of Veterinary Physiology, Biochemistry and Pharmacology, Faculty of Veterinary Medicine, University of Ibadan, Ibadan, Nigeria, **Olubisi O. Soyemi**, Department of Veterinary Surgery and Reproduction, Faculty of Veterinary Medicine, University of Ibadan, Ibadan, Nigeria, and **Ademola A. Oyagbemi**, Department of Veterinary Physiology, Biochemistry and Pharmacology, Faculty of Veterinary Medicine, University of Ibadan, Ibadan, Nigeria.

REFERENCES

Aderibigbe OR, Odetola AA, Oluwole FS, Farombi EO, Onabanjo OO, Jiboku OA. Antioxidant properties of Methanol extract of *Parquetina nigrescens* in Ulcerated rats. International journal of Tropical Medicine 2010; 6(2): 25-29.

Ayoola AO, Akinloye O, Oguntibeju OO, Oke JM, Odetola AA. Antioxidant properties of *Parquetina nigrescens*. Afr. J. Biotechnol. 2011; 10(24): 4920-4925.

Chauhan SS, Ojha S, Mahmood A. Modulation of lipid peroxidation and antioxidant defense systems in rat intestine by subchronic fluoride and ethanol administration Alcohol 2011; 45: 663-672.

Collard CD, Gelman S. Pathophysiology, clinical manifestations, and prevention of ischemia-reperfusion injury. Anesthesiology 2001; 94(6): 1133–1138.

Cornet AD, Kingma SDK, Trof RJ, Wisselink W, Groeneveld ABJ. Hepatosplanchnic ischemia/reperfusion is a major determinant of lung vascular injury after aortic surgery. J Surg Res. (2009) 157(1): 48-54, http://dx.doi.org/10.1016/j.jss.2008.09.021.

Dai J, Mumper RJ. Plant phenolics: Extraction, Analysis and their antioxidant and anticancer properties. Molecules 2010; 15: 7313-7352.

De Arruda MJC, Poggetti RS, Fontes B, Younes RN, Souza AL Jr, Birolini D. Intestinal ischemia/reperfusion induces bronchial hyperreactivity and increases serum TNF-alpha in rats. Clinics. 2006; 61(1):21-8, http://dx.doi.org/10.1590/S1807-59322006000100005.

Farombi, EO, Tahnteng, JG, Agboola, AO, Nwanko, JO, Emerole, GO. Chemoprevention of 2-acetylaminofluorene-induced hepatotoxicity and lipid peroxidation in rats by kolaviron—a Garcina kola seed extract. Food Chem. Toxicol. 2000; 38: 353–541.

Fridovich I. Superoxide dismutases. J. Biol. Chem. 1989; 264: 7761–7764.

Gornal AG, Bardawill JC, David MM. Determination of serum proteins by means of Biuret reaction. J Biol Chem 1949; 177: 751–766.

Gunel E, Caglayan F, Caglayan O, Dilsiz A, Duman S, Aktan M. Treatment of intestinal reperfusion injury using antioxidative agents. J Pediatr Surg 1998; 33(10): 1536–1539.
Habig WH, Pabst MJ, Jacoby WB. Glutathione-S-transferase activity: The enzymic step in mercapturic acid formation. J Biol Chem 1974; 249:130–139.
Higa OH, Parra ER, Ab'Saber AM, Farhat C, Higa R, Capelozzi VL. Protective effects of ascorbic acid pre-treatment in a rat model of intestinal ischemia-reperfusion injury: a histomorphometric study. Clinics 2007; 62(3): 315-20.
Jollow DJ, Mitchell JR, Zampaglione N, Gillette JR. Bro-mobenzene induced liver necrosis: Protective role of glutathione and evidence for 3, 4 bromobenzene oxide as the hepatotoxic metabolite. Pharmacology 1974; 11:151–169.
Köhler HF, DeLucca IMS, Snraglia Neto L. Enteral antioxidants in ischemia/ reperfusion injury in rats. Rev Col Bras Cir. [periódico na Internet] 2011; 38(6). Disponível em URL: http://www.scielo.br/rcbc.
Kolli VK, Abraham P, Isaac B. Alteration in antioxidant defense mechanisms in the small intestines of methotrexate treated rat may contribute to its gastrointestinal toxicity Cancer Therapy 2007; 5: 501-510.
Kolodziejczyk L, Siemieniuk E, Skrzydlewska E. Antioxidant potential of rat liver in experimental infection withFasciola hepatica. Parasitology Research 2005; 96: 367–372.
Masella R, Di Benedetto R, Vari R, Filesi C, Giovannini C. Novel mechanisms of natural antioxidant compounds in biological systems: involvement of glutathione and glutathione-related enzymes. Journal of nutritional Biochemistry. 2005; 16: 577-586.
Mates JM, Perez-Gomez C, Decastro IN. Antioxidant enzymes and human diseases. Clin. Biochem. 1999; 32: 595–603.
Michiels C, Remacle J. Use of the inhibition of enzymatic antioxidant systems to evaluate their physiological importance. Eur J Biochem 1988; 177: 435– 441.
Misra HP, Fridovich I. The role of superoxide anion in the autooxidation of epinephrine and a simple assay for superoxide dismutase. J Biol Chem 1972; 247:3170–3175.
Mojzis j, Hvicova K, Germanova D, Bukovicova D, Mirossay L. Protective effect of quercetin on ischemia/reperfusion-induced gastric mucosal injury in rats. Physiol. Res. 2001; 50(5): 501–506.
Nakamura M, Ozaki M, Fuchinoue S, Teraoka S, Ota K. Ascorbic acid prevents ischemia-reperfusion injury in the rat small intestine. Transpl Int 1997; 10(2): 89–95.
Nieto N, López-Pedrosa JM, Mesa MD, Torres MI, Fernández MI, Ríos A, Suárez MD, Gil A. Chronic Diarrhea Impairs Intestinal Antioxidant Defense System in Rats at Weaning. Digestive Diseases and Sciences, 2000; 45(10): 2044–2050.
Nordstrom G, Seeman T, Hasselgren PO. Beneficial effect of allopurinol in liver ischemia. Surgery; 1985; 97: 679-83.
Nozik-Grayck E, Suliman HB. Piantadosi CA. Extracellular Superoxide dismutase. Int. J. Biochem Cell Biol. 2005; 37: 2466-2471.
Owu DU, Obembe AO, Nwokocha CR, Edoho IE and Osim EE. (2012) Gastric Ulceration in Diabetes Mellitus: Protective role of Vitamin C. ISRN Gastroenterol.
Rice-Evans CA, Miller NJ, Bolwell PG, Bramley PM, Pridham JB. The relative antioxidant activities of plant-derived polyphenolic flavonoids. Free Radic. Res. 1995; 22: 375-383.
Rice-Evans CA, Miller NJ. Paganga, G. Structure-antioxidant activity relationships of flavonoids and phenolic acids. Free Radic. Biol. Med. 1996; 20: 933-956.
Rotruck JT, Pope AL, Ganther HE, Swanson AB, Hafeman DG, Hoekstra WG. Selenium: Biochemical role as a component of glutathione peroxidase. Science 1973; 179:588–590.
Semiz A and Sen A. Antioxidant and chemoprotective properties of Momordica charantia L. (bitter melon) fruit extract African Journal of Biotechnology. 2007; 6 (3): 273-277.
Sido B, Hack V, Hochlehnert A, Lipps H, Herfarth C, Dröge W. Impairment of intestinal glutathione synthesis in patients with inflammatory bowel disease. Gut 1998; 42: 485– 492.
Sies H, Cadenas E. Oxidative stress: damage to intact cells and organs. Philosophical Transactions of the Royal Society B: Biological Sciences 1985, 311: 617–631.
Sinha KA Colorimetric assay of catalase. Anal. Biochem. 1971; 47: 389-394.

Wolff SP. Ferrous ion oxidation in the presence of ferric ion indicator xylenol orange for measurement of hydroperoxides. Method Enzymol 1994; 233:182–189.

Wong DM, Moore RM, Brockus CW. Intestinal ischemia-reperfusion injury in horses: pathogenesis and therapeutics. Compend. Contin. Educ. Vet. 2012; 34(8): E5.

Yamamoto S, Tanabe M, Wakabayashi G, Shimazu M, Matsumoto K, Kitajima M. The role of tumor necrosis factor-alpha and interleukin-1beta in ischemia-reperfusion injury of the rat small intestine. J Surg Res. 2001; 99 (1): 134-141.

Yuan Y, Guo H, Zhang Y, Zhou D, Gan P, Liang DM, Chen JY. Protective effects of L-carnitine on Intestinal Ischemia/reperfusion injury in a rat model J. Clin. Med. Res. 2011; 3(2): 78-84.

Zanoni FL, Benabou S, Greco KV, Moreno ACR, Cruz JWMC, Filgueira FP, *et al*. Mesenteric microcirculatory dysfunctions and translocation of indigenous bacteria in a rat model of strangulated small bowel obstruction. Clinics. 2009; 64(9): 911-9.