ISSN:
Print - 2277 - 0593
Online - 2315 - 7461

Journal of Natural Science, Engineering and Technology

EFFECTS OF METHANOLIC EXTRACT OF *Parquetina nigrescens* ON BACTERIAL TRANSLOCATION IN WISTAR RATS SUBJECTED TO EXPERIMENTAL INTESTINAL ISCHEMIA-REPERFUSION INJURY

***[1]O.A. MAKINDE, [1]A.K. ODUNEYE, [2]A.O. ADEYEYE, [3]E.O. OMOSHABA, [4]A.M. JOODA**

[1]Department of Veterinary Surgery and Theriogenology, Federal University of Agriculture, Abeokuta, P M B 2240, Alabata Road, Abeokuta, Ogun State.
[2]Kentucky State University, Frankfort, KY 40601, USA
[3]Department of Veterinary Microbiology, Federal University of Agriculture, Abeokuta.
[4]Veterinary Teaching Hospital, Federal University of Agriculture, Abeokuta.
***Corresponding Author**: makindeao@funnab.edu.ng **Tel:** +234-803-805-8217

ABSTRACT

Intestinal ischemia-reperfusion injury (IRI) can cause bacterial translocation (BT) and systemic complications. This study evaluated the protective effects of *Parquetina nigrescens* methanolic extract (MEPN) against BT following intestinal IRI. Twenty-four male Wistar rats (180g ± 15g) were randomized into four groups: Group A (negative control, 0.9% saline for 7 days), Group B (MEPN 1000 mg/kg for 7 days without IRI), Group C (MEPN 1000 mg/kg for 7 days prior to IRI), and Group D (ischemic preconditioning: 15 minutes ischemia/15 minutes reperfusion, followed by 30 minutes ischemia/24 hours reperfusion). IRI was induced by superior mesenteric artery clamping for 45 minutes followed by 24-hour reperfusion. All rats were sacrificed by cervical dislocation after 24 hours reperfusion. Bacterial analysis was performed on liver, spleen, lung, terminal ileum, and blood samples using standard microbiological techniques. Data were analyzed using one-way ANOVA. Significantly different mean values were separated using Duncan's multiple range test at $p \leq 0.05$. Multiple bacterial species were isolated, including *Bacillus*, *Staphylococcus saprophyticus*, *Enterococcus*, *Klebsiella*, *E. coli*, *Micrococcus luteus*, and *Citrobacter freundi*. Group A showed no bacterial growth. Group B demonstrated bacterial growth in liver ($4.3\times10^6\pm9.7\times10^6$ CFU) and lung ($1.5\times10^7\pm1.9\times10^7$ CFU). Group C exhibited the highest bacterial loads across all organs, with blood showing $6.0\times10^6\pm1.3\times10^7$ CFU. Group D showed marked reduction with only liver colonization ($6.4\times10^5\pm1.4\times10^6$ CFU), representing 98.5% reduction compared to Group C. MEPN at 1000 mg/kg failed to provide protection against IRI-induced bacterial translocation, while ischemic preconditioning demonstrated significant protective effects.

Keywords: Superior mesenteric artery, ischemic preconditioning, Enterobacteria, reperfusion, herbal medicine, sepsis

INTRODUCTION

Intestinal ischemia-reperfusion (IR) injury is a serious pathological condition that can result from various medical or surgical diseases such as acute mesenteric ischemia, intestinal obstruction, incarcerated hernia, septic shock, thermal injury to the skin; and certain surgical procedures such as small intesti-

nal transplantation, cardiopulmonary bypass or abdominal aortic aneurysm (Yuan *et al*., 2011; Wang *et al*., 2011). IR disrupts nutrient absorption and damages barrier integrity in the intestine, as well as leads to bacterial translocation (Carden and Granger, 2000; Collard and Gelman, 2001). The gastrointestinal tract has been described as one of the organs most sensitive to ischemia and reperfusion (Mojiis *et al*., 2001); this could be due to the fact that intestines are composed of labile cells that are easily injured by episodes of ischemia.

Bacterial translocation involves the passage of viable bacteria from the intestinal lumen through the epithelial mucosa to normally sterile tissues such as mesenteric lymph nodes and other extraintestinal sites. This process occurs when the normal barrier function of the intestinal epithelium is compromised, allowing bacteria to migrate from the gut to other organs or the bloodstream. Bacterial translocation can occur following ischemia reperfusion injury and may lead to systemic infections and sepsis (Tóth *et al*., 2013). Prevention and management of bacterial translocation are of paramount importance to minimize morbidity and mortality associated with intestinal ischemia reperfusion injury.

Oxidative stress mediators such as reactive oxygen species, polymorphonuclear neutrophils and nitric oxide contribute to intestinal IR injury and bacterial translocation by damaging the intestinal epithelial barrier, increasing intestinal permeability, and disrupting the normal gut microbiota balance (Mallick *et al*., 2004; Yang *et al*., 2013). Several interventions have been shown to have protective effects against these conditions, including antioxidants and ischemic preconditioning or pharmacological conditioning techniques. Phytochemical compounds found in medicinal plants have also shown antimicrobial effects, which can inhibit the adhesion of pathogenic bacteria to the intestinal mucosa. The use of phytochemicals to reduce the pathogenic effect of intestinal bacteria is becoming more frequent due to the increase of antimicrobial resistance to antibiotics (Jakhetia *et al*., 2010).

One of such medicinal plant is African parquetuna (*Parquetina nigrescens* (Afzel.) Bullock), which is commonly used in traditional medicine in West Africa and has various therapeutic properties including antioxidant, anti-inflammatory, and antimicrobial effects (Aderibigbe *et al.,* 2011; Owoyele *et al.,* 2011). Although *Parquetina nigrescens* has been reported to possess antioxidant (Aderibigbe *et al.,* 2011; Fadeyemi *et al.,* 2016; Akinrinde *et al*., 2018) and anti-inflammatory properties (Owoyele *et al.,* 2009; Owoyele et al., 2011), its potential for treating bacterial translocation resulting from intestinal IR injury has not been investigated. The mechanism of bacterial translocation involves disruption of the intestinal epithelial barrier, alterations in gut microbiota, and compromised immune function. It has been shown that intestinal tissues are significantly protected against ischemia-reperfusion injury by ischemic preconditioning (IPC), which is a protective mechanism that was initially identified in cardiac tissue (Murry *et al*., 1986; Hotter *et al*., 1996). IPC involves subjecting tissues to brief periods of ischemia and reperfusion to build tolerance against subsequent prolonged ischemic episodes (Cocorullo *et al*., 2004). IPC works in a number of ways, but in the small intestine, it appears to stop post-ischemic leukocyte adhesion by maintaining the bioavailability of nitric oxide (an endogenous anti-adhesive agent), and suppressing the expression of P-selectin (an endothelial-

expressed adhesive molecule) (Korthuis *et al*., 1997). Notably, research has demonstrated that intestinal ischemic preconditioning not only maintains intestinal morphology but also dramatically reduces bacterial translocation (Ozban *et al*., 2002; (Silva *et al*., 2024) through the preservation of tight junction proteins, the integrity of the epithelial barrier, and the inhibition of the inflammatory cascade brought on by IR damage. Remote ischemic preconditioning (RIPC), which applies brief ischemia to distant organs or limbs, has also been shown to lessen mucosal damage, and local IPC has proven effective in protecting intestinal tissues (Bikić *et al*. 2019). However, the need for exact timing, possible risks associated with controlled ischemia in critically ill patients, and individual response variations hinder the clinical application of IPC. In order to provide comparable levels of protection without the risk of controlled ischemia, researchers are now investigating alternative protective strategies like pharmacological agents and natural compounds.

The aim of this study was to explore the effects of the methanolic extract of *Parquetina nigrescens* on bacterial translocation, in a rat model compared to ischemic preconditioning.

MATERIALS AND METHODS

Animal management and experimental design

This study was conducted in full compliance with international guidelines for the care and use of laboratory animals (National Research Council, 2011). Twenty-four (24), male, Wistar rats with average weight of 180g ± 15g were obtained and allowed to acclimatize for seven days at the laboratory animal house of the Department of Veterinary Pharmacology and Toxicology, Federal University of Agriculture, Abeokuta, before the commencement of the experiment. The rats were kept in individual cages in a well-ventilated room and fed with commercially formulated feed (Top feed®, Premier Feeds Mill Nigeria Limited, Ibadan, Nigeria) and clean water *ad libitum* until 12 hours before the procedure. The animals were subjected to natural light-dark cycle under standard room temperature.

They were randomly assigned into four (4) groups viz:

i. Group A (Negative Control): Animals received no ischemia/reperfusion (I/R) injury and were administered 1ml of 0.9% normal saline solution orally for 7 consecutive days.

ii. Group B: Animals received no ischemia/reperfusion (I/R) injury and were administered 1000 mg/kg of methanolic extract of *P. nigrescens* orally for 7 consecutive days.

iii. Group C: Animals were subjected to I/R injury and pretreated with 1000 mg/kg of methanolic extract of *P. nigrescens* orally for 7 consecutive days before IRI induction.

iv. Group D: Animals were subjected to I/R injury with ischemic preconditioning (IPC) of brief episodes of ischemia and reperfusion for 15 minutes each and then prolonged ischemia for 30 minutes and reperfusion for 24 hours.

The dosage of *Parquetina nigrescens* (1000 mg/kg) and preconditioning time were chosen according to previous works by Akinrinmade *et al*. (2016) and Aldo *et al*. (2013), respectively.

Plant Collection and Extraction

Fresh leaves of *Parquetina nigrescens* were obtained within the premises of Federal University of Agriculture, Abeokuta and authenticated in the Department of Pure and Applied Botany of the same university. The

leaves were washed and air dried in the absence of sunlight (for 4 weeks), blended, and grounded into a 40-mesh powdered size. 300 g of the dried, powdered leaves of *Parquetina nigrescens* was soaked in 2000 ml of pure methanol inside a glass cylinder for 72 hours, at room temperature (27 ±2 °C). The solvent containing the crude extract was then collected, filtered and concentrated using a Rotary Evaporator at 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 26.891g (percentage yield was 8.96%). It was stored at 4°C in an airtight container throughout the period of the experiment. Distilled water was used to dissolve and make up the required volume for administration at a concentration of 100 mg/ml.

Surgical procedure

Following overnight fasting, rats were anaesthetized with intramuscular injections of 70 mg/kg ketamine [Rotexmedica, Trittau, Germany] and 7 mg/kg xylazine [Xylazine 20Inj®, Kepro, Holland] (Struck *et al*., 2014). The rats were maintained under spontaneous breathing to ensure adequate oxygenation throughout the procedure. Once anaesthesia was evident by the loss of reflexes and muscle relaxation, the animals were positioned on a surgical table. Under sterile conditions with proper surgical draping and instrument sterilization, a ventral midline laparotomy incision was made; the abdominal viscera were retracted and wrapped in a gauze soaked in normal saline solution. The superior mesenteric artery was isolated at its origin and occluded immediately distal to the aorta with atraumatic microvascular clamp for 45 minutes (Diniz *et al.,* 2005) in groups C. Ischemia was confirmed by the loss of mesenteric pulsations and blanching of the intestine. After 45 minutes of ischemia, the microvascular clamp was removed and reperfusion was confirmed by the restoration of mesenteric pulsations and return of normal intestinal color, which was maintained for 24 hours. In group D, clamping of the mesenteric artery was done for 15 minutes and reperfusion for 15 minutes, followed by further clamping for 30 minutes, and reperfusion was also maintained for 24 hours. After the procedures, all animals had their abdomens closed routinely with nylon monofilament 3-0 sutures, and observed in individual cages for any complications.

Sample collection

After 24 hours of reperfusion, the animals were euthanized by cervical dislocation. Under sterile conditions, a new laparotomy was performed and blood samples were collected via cardiac puncture into ethylene diamine tetra-acetic acid (EDTA) sample bottles for microbiological culture. Sterile tissue samples of the liver, spleen, lung and terminal ileum were collected into individual sterile sample bottles for quantitative culture in appropriate media for bacterial organisms.

Microbiological examination

Bacterial plate count

1g each of lung, liver, spleen and ileum samples were weighed and placed in a sterile grinding mortal. Tissues were homogenized in 9 mL of normal saline for quantitative cultures. A fivefold serial dilution of the homogenized tissue sample was prepared after pipetting of 9.0 mL of the diluent (normal saline) into each 5 tubes. 0.5 mL of the sample from the last (5th) dilution points of the dilution series was deposited onto the surface of media (MacConkey agar) using a

sterile needle and syringe, after which the plate was gently shaken for easy spread of the sample on the surface of the media. Sterile inoculating wire loop was dipped into the last dilution points of the series and streaked on blood agar. The plates (MacConkey and blood agar) were incubated at 37°C aerobically for 24 hours after which growth was observed. Colonies were counted and expressed as the number of colony-forming units per gram (CFU/g) of tissue homogenate (Isenberg, 1992).

Bacteria identification

Bacterial identification was based on cultural morphology, Gram staining and biochemical characteristics including catalase test, oxidase test, coagulase test, triple sugar ion test, sulfide indole motility test and citrate test (Cheesbrough, 2006). A colony of pure isolate from the counted bacterial plate was streaked on MacConkey agar plate. After 24 hours of incubation at 37°C, isolates were kept in nutrient agar slopes for further biochemical tests and stored in the refrigerator at 4°C (Cheesbrough, 2006). Blood (0.5 mL) samples were also cultured in 4 mL of buffered peptone water for 24 hours at 37° C. These were however sub-cultured on blood agar and MacConkey agar plates.

Statistical analysis

Quantitative data obtained were recorded as mean ± standard error of mean. CFU data collected was subjected to One-way Analysis of variance (ANOVA) followed by Duncan multiple range test to find significance among the means of Colony Forming Units (CFU) recorded using SPSS (Statistical Package for Social Sciences) version 20. Value of $p \leq 0.05$ was considered statistically significant.

RESULTS

Group A (negative control) demonstrated complete absence of bacterial growth in all tissue samples examined (Table 1). Group B (MEPN treatment without IRI) showed bacterial colonization in liver ($4.3\times10^6\pm9.7\times10^6$ CFU) and lung ($1.5\times10^7\pm1.9\times10^7$ CFU) tissues despite the absence of ischemia-reperfusion injury.

Group C (MEPN pretreatment + IRI) exhibited the most extensive bacterial translocation across all organs examined compared to other Groups. Blood samples showed significant bacterial growth ($6.0\times10^6\pm1.3\times10^7$ CFU), while all other groups did not show any growth. Liver samples demonstrated the highest bacterial load ($4.3\times10^7\pm4.3\times10^7$ CFU) compared to Groups B and D. Spleen colonization was observed at $9.4\times10^6\pm2.1\times10^7$ CFU, lung at $2.8\times10^5\pm6.3\times10^5$ CFU, and terminal ileum at $2.9\times10^7\pm3.9\times10^7$ CFU.

Group D (ischemic preconditioning + IRI) showed markedly reduced bacterial translocation compared to Group C, with bacterial growth detected only in liver samples ($6.4\times10^5\pm1.4\times10^6$ CFU). This represented a 98.5% reduction in liver bacterial load compared to the MEPN-treated group (Table 1). Comparative analysis revealed significant differences in bacterial colonization patterns between groups. In liver samples, Group C showed significantly higher CFU compared to both Groups B and D indicating that MEPN pretreatment failed to prevent bacterial translocation. Conversely, Groups B and D showed statistically similar liver bacterial loads, suggesting comparable colonization levels despite different treatment protocols.

In lung samples, Group B demonstrated significantly higher CFU ($1.5\times10^7\pm1.9\times10^7$) compared to Group C ($2.8\times10^5\pm6.3\times10^5$) ($p<0.05$), indicating an unexpected finding where MEPN treatment alone resulted in greater pulmonary bacterial colonization than IRI with MEPN pretreatment (Table 1).

Table 1: CFUs Values in Cultured Tissues from Animals in Groups A, B, C and D

	GROUP A	**GROUP B**	**GROUP C**	**GROUP D**
BLOOD	No Growth	No Growth	$6.0 \times 10^6 \pm 1.3 \times 10^7$	No Growth
LIVER	No Growth	$4.3 \times 10^6 \pm 9.7 \times 10^{6b}$	$4.3 \times 10^7 \pm 4.3 \times 10^{7a}$	$6.4 \times 10^5 \pm 1.4 \times 10^{6b}$
LUNG	No Growth	$1.5 \times 10^7 \pm 1.9 \times 10^{7a}$	$2.8 \times 10^5 \pm 6.3 \times 10^{5b}$	No Growth
SPLEEN	No Growth	No Growth	$9.4 \times 10^6 \pm 2.1 \times 10^7$	No Growth
TERMINAL ILEUM	No Growth	No Growth	$2.9 \times 10^7 \pm 3.9 \times 10^7$	No Growth

NOTE: Data are expressed as mean ± SEM. Values with different superscripts across a row are statistically significant ($p>0.05$).

Microbiological analysis identified ten distinct bacterial species across the tissue samples (Table 2). The isolated organisms included both Gram-positive bacteria (*Bacillus* species, *Staphylococcus saprophyticus*, *Staphylococcus epidermidis*, *Enterococcus* species, *Micrococcus luteus*) and Gram-negative bacteria (*Escherichia coli*, *Klebsiella* species, *Proteus mirabilis*, *Citrobacter freundi*, *Pseudomonas aeruginosa*).

Analysis of bacterial species distribution revealed that *Bacillus* species, *Staphylococcus saprophyticus*, and *Enterococcus* species were the most widely distributed, being isolated from all tissue samples that showed bacterial growth. *Staphylococcus epidermidis* was recovered from all organs except blood samples, while *Proteus mirabilis* was isolated from terminal ileum, liver, and lung tissues.

The terminal ileum demonstrated the highest bacterial diversity, harboring 10 different species (37% of total bacterial isolates identified), followed by liver and lung tissues with 7 species each (26% each). Spleen samples showed intermediate diversity with 5 species (19%), while blood samples contained the lowest diversity (11%) with 3 species (Table 2).

Table 2: Profile of bacteria organisms isolated on blood and MacConkey agars from tissue samples of rats used in this study

SAMPLES	ISOLATED BACTERIA
BLOOD	*Bacillus species, Staphylococcus saprophyticus, Enterococcus*
LIVER	*Bacillus species, Staphylococcus saprophyticus, Enterococcus, Proteus mirabilis, Staphylococcus epidermidis, Klebsiella, Escherichia coli*
LUNG	*Bacillus species, Staphylococcus saprophyticus, Enterococcus, Proteus mirabilis, Staphylococcus epidermidis, Micrococcus luteus, Citrobacter freundi*
SPLEEN	*Bacillus species, Staphylococcus saprophyticus, Enterococcus, Micrococcus luteus, Staphylococcus epidermidis*
TERMINAL ILEUM	*Bacillus species, Escherichia coli, Enterococcus, Proteus mirabilis, Staphylococcus epidermidis, Klebsiella, Micrococcus luteus, Staphylococcus saprophyticus, Citrobacter freundi, Pseudomonas aeruginosa*

DISCUSSION

The findings of this study indicate that the methanolic extract of *Parquetina nigrescens* does not provide protection against bacterial translocation induced by intestinal ischemia-reperfusion injury in rats. This assertion is based on the observation that Group C, which received MEPN pretreatment prior to IRI, showed significant bacterial growth in multiple organs, compared to the control groups. The study confirms that intestinal IRI leads to bacterial translocation, as evidenced by the isolation of various bacterial species from extraintestinal organs in Group C, which received MEPN pretreatment prior to IRI. The terminal ileum was found to be the most affected tissue in terms of bacterial translocation, which is consistent with previous findings that bacterial load increases along the length of the intestine (Baumgart and Dignass, 2002). This finding aligns with the anatomical and physiological characteristics of the terminal ileum, which has a higher bacterial load compared to other parts of the small intestine.

The ischemic preconditioning group (Group D) showed better outcomes with reduced bacterial translocation compared to Group C that were pretreated with 1000 mg/kg MEPN orally for 7 days, prior to induction of intestinal IRI, suggesting that ischemic preconditioning may be an effective intervention in preventing this pathological process. This supports the findings of Aldo *et al.* (2013), who demonstrated that 15-minutes of ischemic preconditioning attenuated bacterial translocation.

The 1000 mg/kg dosage of the methanolic extract of *Parquetina nigrescens* used in this study was based on previous research by Akinrinmade *et al.* (2016). While Akinrinmade *et al.* demonstrated the significant antioxidant properties of MEPN, the current study suggests that these antioxidant properties may not be sufficient to prevent bacterial translocation under the conditions tested. The lack of efficacy may be due to the complex pathophysiology of bacterial translocation, which involves multiple factors beyond oxidative stress.

Failure of MEPN to prevent bacterial translocation in this study suggests that its phytochemical constituents may not adequately protect tight junction proteins, including

claudins, occludins, and zonula occludens-1, which are particularly vulnerable to ischemic insult and subsequent inflammatory mediators (Suzuki, 2013). Research by Grootjans *et al.* (2016) demonstrated that effective prevention of bacterial translocation requires not only antioxidant activity but also specific protection of tight junction integrity, which may exceed the therapeutic scope of MEPN at the tested concentration. While *P. nigrescens* has demonstrated anti-inflammatory properties in other contexts (Owoyele *et al.*, 2011), the magnitude of inflammatory response during severe IRI may overwhelm the plant's anti-inflammatory capacity. Studies by Yang *et al.* (2013) has indicated that successful prevention of IRI-induced bacterial translocation requires potent anti-inflammatory interventions that can significantly attenuate the cytokine cascade, a threshold that MEPN may not have achieved in this study. Failure of MEPN to provide protection also suggests limited efficacy in addressing microvascular dysfunction, which may require more targeted interventions such as phosphodiesterase inhibitors or direct endothelial protective agents (Vollmar *et al.*, 2011).

While *P. nigrescens* possesses antioxidant properties, the oxidative stress associated with IRI extends beyond simple reactive oxygen species (ROS) generation. The antioxidant capacity of MEPN may be insufficient to counteract the intense oxidative stress generated by massive neutrophil infiltration, particularly during the reperfusion phase when neutrophil recruitment is highest (Yasuhara *et al.*, 2003).

IRI significantly alters the composition and function of the gut microbiome, leading to dysbiosis that promotes pathogenic bacterial overgrowth and translocation (Haak *et al.*, 2012). While some phytochemicals demonstrate prebiotic properties, the acute nature of IRI-induced dysbiosis may require more immediate and potent interventions to restore microbial balance. Failure of MEPN to prevent bacterial translocation may reflect its limited ability to rapidly modulate the gut microbiome under severe pathological conditions (Cresci and Bawden, 2015).

IRI induces significant alterations in both innate and adaptive immune responses that compromise the host's ability to contain bacterial translocation (Mallick *et al.*, 2004). Failure of MEPN to prevent bacterial translocation may reflect its inability to adequately support immune function during the acute phase of IRI, when rapid restoration of immune competence is crucial for preventing bacterial dissemination.

The bacterial species isolated in this study, including *Proteus mirabilis*, *Escherichia coli*, *Klebsiella*, *Enterococcus*, and *Citrobacter freundii*, are consistent with previous findings by Abdulkadir *et al.* (2003). Additional species such as *Staphylococcus* species, *Micrococcus luteus*, and *Pseudomonas aeruginosa* were also identified and could potentially contribute to septic complications in IRI patients.

The unexpected bacterial growth in Group B (which received MEPN without IRI) suggests possible post-mortem contamination or handling artifacts, as these animals were not subjected to surgical procedures that would compromise intestinal barrier function (Quinn *et al.*, 2011), potential extract-related effects (Becker *et al.*, 2014), and methodological considerations (Sedrish *et al.*, 2011).

This bacterial growth in Group B which received MEPN without IRI does not appear

to compromise the primary conclusion of this study regarding MEPN's lack of efficacy against IRI-induced bacterial translocation. The bacterial growth patterns in Group C of IRI + MEPN were substantially different from those in Group B which received MEPN without IRI, both in terms of bacterial load and organ distribution. Specifically, Group C of IRI + MEPN, showed significant bacterial growth in the terminal ileum and blood, which were absent in Group B which received MEPN without IRI, suggesting that the IRI-induced translocation observed in Group C of IRI + MEPN, represents a distinct pathological process rather than simple contamination. The absence of bacterial growth in the terminal ileum of the Negative control, treated with 1ml of 0.9% of normal saline orally for 7 days (Group A) and the ischemic preconditioning (Group D) may be due to the preservation of normal intestinal barrier function and the sampling technique used, which may not have captured the normal commensal microflora.

CONCLUSION

The findings of this study indicate that under the experimental conditions tested, MEPN at the studied dose and duration not only fails to protect against IRI-induced bacterial translocation but may potentially compromise intestinal barrier integrity even in the absence of ischemic injury. The study confirms that ischemic preconditioning remains a more effective intervention for preventing bacterial translocation following intestinal IRI.

RECOMMENDATIONS

Given the unexpected bacterial growth observed in animals treated with MEPN alone (Group B), future studies should: Investigate the dose-response relationship of MEPN to determine if lower doses might be protective without compromising intestinal barrier function; Examine different extraction methods and administration routes that might improve efficacy; Include additional control groups to rule out contamination and better understand the extract's effects on normal intestinal physiology; Conduct histopathological examination of intestinal tissues to assess barrier integrity; and Consider combination therapies that might enhance the protective effects while minimizing potential adverse effects.

While traditional herbal remedies have shown potential therapeutic benefits in various conditions, this study suggests that *P. nigrescens* extract at the tested parameters may not be suitable for preventing IRI-related bacterial translocation and requires further optimization before clinical consideration.

REFERENCES

Abdulkadir, B., Selma, G., Kadir, C. K., Isin, S., Likay, G., and Sakrak, O. 2003. Bacterial translocation after partial hepatic resection under ischemia and reperfusion in rats: Incidence and time course. *Turkish Journal of Medical Sciences*, 33(3): 135-140.

Aderibigbe, O. R., Odetola, A. A., Oluwole, F. S., Farombi, E. O., Onabanjo, O. O., and Jiboku, O. A. 2011. Antioxidant properties of methanol extract of Parquetina nigrescens in ulcerated rats. *International Journal of Tropical Medicine*, 6(2): 25-29.

Akinrinde, S. A., Makinde, A. O., and Akinrinmade, J. F. 2018. Flavonoid-rich extract of Parquetina nigrescens prevents intestinal ischemia-reperfusion injury in Wistar rats via modulation of inflammatory cytokines and antioxidant defense. *Tropical Veterinarian*, 36(3): 151-165.

Akinrinmade, F. J., Akinrinde, A. S., Soyemi, O. O., and Oyagbemi, A. A. 2016. Antioxidant potential of the methanol extract of Parquetina nigrescens mediates protection against intestinal ischemia-reperfusion injury in rats. *Journal of Dietary Supplements*, 13(4): 420-432.

Aldo da Cunha, M., Irami, A., Mariana, L. T., Caroline de Vasconcelos, S., Daniel, T. J., and Amalia, C. M. R. 2013. Ischemic preconditioning in different times and its effect on bacterial translocation induced by intestinal ischemia and reperfusion in rats. *Revista do Colegio Brasileiro de Cirurgioes*, 40(1): 3-6.

Baumgart, D. C., and Dignass, A. U. 2002. Intestinal barrier function. *Current Opinion in Clinical Nutrition and Metabolic Care*, 5(6): 685-694.

Becker, S., Oelschlaeger, T. A., Wullaert, A., Vlantis, K., Pasparakis, M., Wehkamp, J., Berger, J., Wittkopf, N., Bode, K. A., Jahn, D., Vieth, M., Billmeier, U., Zundler, S., Mundt, P., Wittkopf, A., Neurath, M. F., and Schaeffeler, E. 2014. Bacteria regulate intestinal epithelial cell differentiation factors both in vitro and in vivo. *PLoS One*, 9(8); e106611.

Bikić, S., Gmajnić, R., Prvulović, M., Srdić, B., Ilić, A., Bogosavljević, A., Čemerikić, N., Stanimirović, J., Milošević, I., Srbovan, D., Stojanović, M., and Golubović, M. 2019. Remote ischemic preconditioning attenuates intestinal mucosal damage: Insight from a rat model of ischemia–reperfusion injury. *Journal of Translational Medicine*, *17*(1), Article 138.

Carden, D. L., and Granger, D. N. 2000. Pathophysiology of ischemia-reperfusion injury. *Journal of Pathology*, 190(3): 255-266.

Cheesbrough, M. 2006. *District laboratory practice in tropical countries* (2nd Ed.). Cambridge University Press.

Cocorullo, G., Tutino, R., Falco, N., Licari, L., Orlando, G., Fontana, T., Raspanti, C., Salamone, G., Scerrino, G., and Gulotta, G. 2014. Ischemic preconditioning protects intestine from prolonged ischemia. *Transplantation Proceedings*, *46*(11), 3756-3759.

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

Cresci, G., and Bawden, E. 2015. Gut microbiome: What we do and don't know. *Nutrition in Clinical Practice*, 30(6): 734-746.

Diniz, S. O. F., Barbosa, A. J., Araujo, I. D., Nelson, D. L., Machado, A. S., Filho, M. B., and Cardoso, V. N. 2005. Assessment of bacterial translocation in obstructive jaundice using Tc-99m Escherichia coli. *Brazilian Archives of Biology and Technology*, 48(1): 45-49.

Fadeyemi, J. A., Akinleye, S. A., Olubisi, O. S., and Ademola, A. O. 2016. Antioxidant potential of the methanol extract of Parquetina nigrescens mediates protection against intestinal ischemia-reperfusion injury in rats. *Journal of Dietary Supplements*, 13(4): 420-432.

Grootjans, J., Thuijls, G., Verdam, F., Derikx, J. P., Lenaerts, K., and Buurman, W. A. 2016. Non-invasive assessment of barrier function and mucosal integrity in hu-

mans. *World Journal of Gastrointestinal Pathophysiology*, 1(3): 99-105.

Haak, B. W., Westendorp, W. F., van Engelen, T. S., Brands, X., Brouwer, M. C., Vermeij, J. D., and Wiersinga, W. J. 2012. Disruptions of anaerobic gut bacteria are associated with stroke and post-stroke infection: A prospective case-control study. *Translational Stroke Research*, 12(4): 581-592.

Hotter, G., Closa, D., Prados, J., Fernández-Cruz, L., Prats, N., Gelpí, E., and Roselló-Catafau, J. 1996. Intestinal preconditioning is mediated by a transient increase in nitric oxide. *Biochemical and Biophysical Research Communications*, *222*(1), 27-32.

Isenberg, H. D. 1992. *Clinical microbiology procedures handbook* (1st ed.). ASM Press.

Jakhetia, V., Patel, R., Khatri, P., Pahuja, N., Garg, S., Pandey, A., and Sharma, S. 2010. Cinnamon: A pharmacological review. *Journal of Advanced Scientific Research*, 1(2): 19-23.

Korthuis, R. J., Granger, D. N., Townsley, M. I., and Taylor, A. E. 1985. The role of oxygen-derived free radicals in ischemia-induced increases in canine skeletal muscle vascular permeability. *Circulation Research*, *57*(4), 599-609.

Mallick, I. H., Yang, W., Winslet, M. C., and Seifalian, A. M. 2004. Ischemia-reperfusion injury of the intestine and protective strategies against injury. *Digestive Diseases and Sciences*, 49(9): 1359-1377.

Mojiis, J., Hviseova, K., Germanova, D., Bukovieova, D., and Mirossay, L. 2001. Protective effect of quercetin on ischemia/reperfusion-induced gastric mucosal injury in rats. *Physiological Research*, 50(5); 501-506.

Murry, C. E., Jennings, R. B., and Reimer, K. A. 1986. Preconditioning with ischemia: A delay of lethal cell injury in ischemic myocardium. *Circulation*, *74*(5), 1124-1136.

National Research Council. 2011. *Guide for the care and use of laboratory animals* (8th ed.). National Academies Press.

Owoyele, B. V., Abdulrazaq, B. N., Idris, A. O., Lukuman, A. O., and Ayodele, O. S. 2009. Studies on the analgesic, anti-inflammatory and antipyretic effects of Parquetina nigrescens leaf extract. *Journal of Ethnopharmacology*, 122(1), 86-90.

Owoyele, B. V., Oyelowo, O. T., Biliaminu, S. A., Alaran, O. N., Alimi, S. A., and Saliu, R. S. 2011. Haematological and biochemical studies on P. nigrescens root extract in albino rats. *Journal of Applied Pharmaceutical Science*, 1(10), 176-179.

Ozban, M., Aydin, C., Cevahir, N., Yenisey, C., Birsen, O., Goktas, S., and Bilgic, L. 2002. Intestinal ischemic preconditioning protects the intestine and reduces bacterial translocation. *Shock*, *18*(5), 476-480.

Quinn, P. J., Markey, B. K., Leonard, F. C., Hartigan, P., Fanning, S., and Fitzpatrick, E. 2011. *Veterinary Microbiology and Microbial Disease* (2nd ed.). Wiley-Blackwell.

Sedrish, S. A., Keuler, N. S., Barger, A. M., Biller, D. S., Fenwick, B., Liepnieks, M., and Constable, P. D. 2011. Comparison of the sensitivity and specificity of bacterial culture of samples obtained by use of ultrasound-guided fine-needle aspiration and ultrasound-guided tissue-core biopsy for di-

agnosis of septic arthritis in dogs. *Journal of the American Veterinary Medical Association*, 238(7): 909-917.

Silva, M. A., Carneiro, F. P., Ferreira, D., Moreno, C., Ramalho, F., Silva, E., Brocardo, P. S., Zanoni, F. L., and Brocardo, G. A. 2024. Effects of remote ischemic preconditioning on early markers of intestinal injury in experimental hemorrhage in rats. *Scientific Reports*, *14*(1), Article 12872.

Struck, M. B., Andrutis, K. A., Ramirez, H. E., and Battles, A. H. 2014. Effects of a short-term fast on ketamine-xylazine anesthesia in rats. *Journal of the American Association for Laboratory Animal Science*, 53(3): 344-348.

Suzuki, T. 2013. Regulation of intestinal epithelial permeability by tight junctions. *Cellular and Molecular Life Sciences*, 70(4): 631-659.

Tóth, S., Jr., Pekárová, T., Varga, J., Tomecková, V., Tóth, S., and Lakyová, L. 2013. Trehalase as a possible marker of intestinal ischemia-reperfusion injury. *Acta Biochimica Polonica*, 60(3): 411-416.

Vollmar, B., Glasz, J., Leiderer, R., Post, S., and Menger, M. D. 2011. Hepatic microcirculatory perfusion failure is a determinant of liver dysfunction in warm ischemia-reperfusion. *American Journal of Pathology*, 145 (6): 1421-1431.

Wang, B., Huang, Q., Zhang, W., Li, N., and Li, J. 2011. Lactobacillus plantarum prevents bacterial translocation in rats following ischemia and reperfusion injury. *Digestive Diseases and Sciences*, 56(11): 3187-3194.

Yang, X., Bai, H., Cai, W., Li, J., Zhou, Q., and Wang, Y. 2013. Lycium barbarum polysaccharides reduce intestinal ischemia/reperfusion injuries in rats. *Chemico-Biological Interactions*, 204(3): 166-172.

Yasuhara, S., Perez, M. E., Kanakubo, E., Yasuhara, Y., Shin, Y. S., Kaneki, M., Fujita, T., and Martyn, J. A. 2003. Skeletal muscle apoptosis after burns is associated with activation of proapoptotic signals. *American Journal of Physiology-Endocrinology and Metabolism*, 285(5), E1005-E1012.

Yuan, Y., Guo, H., and Zhang, Y. 2011. Protective effects of L-carnitine on intestinal ischemia/reperfusion injury in a rat model. *Journal of Clinical Medicine Research*, 3(2): 78-84.

(Manuscript received: 7th December, 2023; accepted: 28th October, 2025).