Access this Article online
Quick Response Code:

Website:
jecajournal.com
Doi:
doi.org/10.4314/jeca.v20i2.3

Original Article

Investigating the Effects of Aqueous *Zingiber officinale* Rhizome Extract on CCL_4-induced Liver Alterations in Wistar Rats

[1]Momodu, O.I.; [1]Idemudia, O.U.

Abstract

BACKGROUND AND AIM: The medicinal value of plants has long been recognized with numerous drugs derived from them proving essential in disease treatment. Accordingly, this study investigated the effects of aqueous *Zingiber officinale* rhizome extract (AZOR) against carbon tetrachloride (CCl_4)-induced liver alterations in Wistar rats.

METHODOLOGY: Twenty adult Wistar rats were assigned into a control Group (A) and three treatment Groups (B-D) containing five rats each. Rats in group B received 200 mg/kg body weight (BW) of AZOR; Rats in treatment groups C and D were administered with an intraperitoneal injection of 1 ml/ kg BW of 30% CCl_4/olive oil mixture every 72 h for 14 days, however, rats in Group C were treated daily with 200 mg/kg BW of AZOR. Thereafter, the rats were sacrificed and blood samples were collected to assay for aspartate aminotransferase (AST), alanine aminotransferase (ALT), alkaline phosphatase (ALP), total bilirubin (TB), Superoxide dismutase (SOD), Catalase (CAT), Glutathione Peroxidase (GPx) and Malondialdehyde (MDA). Histological analyses were conducted to assess the effects of these treatments.

RESULTS: Findings revealed a significant increase in AST, ALT, ALP, TB, and MDA as well as a significant decrease in SOD, CAT and GPx in the group treated with CCl_4 alone, indicative of liver damage. Histological findings showed severe steatosis in the group treated with CCl_4 alone. However, treatment with AZOR attenuated these adverse effects, suggesting a protective effect of the extract against CCl_4-induced hepatotoxicity.

CONCLUSION: Taken together, the hepatoprotective potential of AZOR against CCl_4 could be attributed to the antioxidant properties of the plant.

Keywords:
Zingiber officinale, Hepatoprotective, Carbon tetrachloride, Liver enzymes, Wistar rats

INTRODUCTION

The utilization of natural sources for medicinal purposes dates back thousands of years, with numerous modern drugs derived from such sources proving crucial in disease treatment (Enogieru and Momodu, 2021; Enogieru *et al.,* 2018). Traditional knowledge surrounding medicinal plants has long been instrumental in the quest for new remedies, offering cost-effective, readily available solutions often in the form of simple preparations (Park and Pezzutto, 2002). With their array of active chemical constituents, medicinal plants are often likened to "Chemical Goldmines," providing compounds vital for human and animal health not easily synthesized in laboratories. Of the approximately 250,000 higher plant species globally, more than 80,000 possess medicinal properties (Joy *et al*., 1998).

One such plant, *Zingiber officinale* Roscoe, commonly known as ginger, holds significant medicinal, nutritional, and ethnomedical value and is widely used worldwide for its diverse therapeutic properties (Grzanna *et al*., 2005). Belonging to the Zingiberaceae family, which is among the largest monocotyledonous families in India, ginger has a rich history of traditional use in various medicinal systems for treating ailments such as nausea, inflammation, and pain (Jain and Prakash, 1995). With numerous biological properties attributed to extracts from the Zingiberaceae family, including antimicrobial and antioxidant effects, ginger and its relatives have garnered attention for their potential health benefits (Sacchetti *et al*., 2005).

Submitted: 5th March, 2024
Revised: 23rd March, 2024
Accepted: 27th March, 2024
Published: 15th April, 2024

[1]*Department of Anatomy, School of Basic Medical Sciences, University of Benin, Edo State, Nigeria.*

Address for Correspondence:
Idemudia, O.U.
Department of Anatomy, School of Basic Medical Sciences, University of Benin, Edo State, Nigeria.
+2348023635 289
eghosa.idemudia@uniben.edu

This is an open access journal, and articles are distributed under the terms of the Creative Commons Attribution-Non Commercial-Share Alike 4.0 License, which allows others to remix, tweak, and build upon the work non-commercially, as long as appropriate credit is given and the new creations are licensed under the identical terms.

For reprints contact: jecajournal@gmail.com

How to cite this article: Momodu, O.I. and Idemudia, O.U. Investigating the Effects of Aqueous *Zingiber officinale* Rhizome Extract on CCL_4-induced Liver Alterations in Wistar Rats. J Exp Clin Anat 2023; 20(2):15-19. doi.org/10.4314/jeca.v20i2.3

Originating in Southeast Asia, ginger has been cultivated for millennia for both its culinary and medicinal purposes, with India and China currently serving as major global suppliers (Vasala, 2004).

Despite its many applications, certain chemicals pose risks to human health and the environment, such as carbon tetrachloride (CCl_4). This compound, once widely used in various industries, has since been phased out due to its toxic nature, particularly its detrimental effects on the liver and central nervous system (Lunn *et al*., 2022). When metabolized by the liver, CCl_4 leads to hepatotoxicity, impairing liver function through inflammation and cellular damage (Seifert *et al*., 1994). Against this backdrop, the present study aims to investigate the effects of *Zingiber officinale* against CCl_4-induced alterations in the liver of Wistar rats, focusing on alterations in antioxidant profiles, enzymes and histological changes.

Materials and Methods

Plant Material

Aqueous extraction of *Zingiber officinale* was done using the Freeze-drying method (Enogieru and Omoruyi, 2022). Briefly, the rhizomes were chopped into little bits and allowed to dry at room temperature. The dried rhizomes were pounded using a wooden mortar and pestle and milled into fine powder in an electric blender. 500 g of the powder was soaked in 2 litres of distilled water for 24 hours. The mixture was filtered with Whatman filter paper No 42 (125 mm) and the residue was separated from the filtrate. The filtrate was concentrated at the National Centre for Energy and Environment at the University of Benin, Benin City.

Care of Experimental Animals

Twenty (20) adult male Wistar rats weighing between 150 g and 170 g were used for this experiment. Care and management of animals were carried out in accordance with the guidelines for the care and use of laboratory animals (NRC, 2010). The animals were allowed to acclimatize for two weeks before commencement of the experiment.

Treatment Regimen

The rats were randomly assigned into a Control group (A) and three treatment groups (B-D) containing five (5) animals each. Rats in group A received distilled water. Rats in group B received 200 mg/kg body weight (BW) of aqueous *Zingiber officinale* rhizome extract. Rats in the treatment groups C and D were administered with intraperitoneal injection of 1 ml/ kg BW of 30% CCl_4/olive oil mixture every 72 h for 14 days, however, rats in Group C were treated daily with 200 mg/kg BW of aqueous *Zingiber officinale* rhizome extract.

Animal Sacrifice and Evaluation of Biochemical Parameters

At the end of the experimental period, rats were sacrificed by cervical dislocation and blood samples were collected, by Cardiac puncture, in plain bottles for determination of liver enzymes (Aspartate aminotransferase (AST), Alkaline phosphatase (ALP), Alanin transaminase (ALT) and Total bilirubin (TB) as previously reported (Enogieru *et al*., 2015a). 0.5 g of the liver tissue was macerated in 5 ml of distilled water for the determination of antioxidant enzymes (Superoxide Dismutase (SOD), Catalase (CAT), Glutathione Peroxidase (GPx) and Malondialdehyde (MDA) as previously reported (Enogieru and Momodu, 2022).

Histological Assessment

Following appropriate fixation (10% buffered formal saline) of the liver for seventy-two hours, processing through the paraffin wax embedding and the hematoxylin and eosin staining methods were carried out as previously described (Drury and Wallington, 1980).

Statistical analysis

Statistical analysis was done using the IBM Statistical Package for Social Sciences, Version 23 (manufactured by International Business Corporations {IBM}; released in 2015). All values were presented in Mean ± standard error of the mean for all groups, significance was determined using one-way ANOVA followed by Turkey's multiple comparisons post-hoc test and a value of $P<0.05$ was taken as statistically significant.

Results

Effect of treatment on liver function enzymes

Table 1 shows the results obtained from the liver function test AST, ALP, ALT and TB. There was a significant increase ($P<0.05$) in serum AST, ALT, ALP and TB levels in rats treated with CCL_4 only compared with control. However, there was a significant decrease ($P<0.05$) in serum AST, ALT, ALP and TB levels in rats cotreated with ginger when compared to CCL_4 only. There was no significant difference ($P>0.05$) in the levels of AST, ALT, ALP and TB in the group treated with ginger only when compared to control.

Effect of treatment on antioxidant enzymes

Table 2 shows the results obtained from tissue antioxidant enzymes and lipid peroxidation. There was a significant decrease ($P<0.05$) in the level of SOD, CAT, and GPx as well as a significant increase ($P<0.05$) in the level of MDA in rats treated with CCL_4 only compared to control. However, there was a significant increase ($P<0.05$) in the level of SOD, CAT, and GPx as well as a decrease in the level of MDA in rats cotreated with ginger when compared to CCL_4 only. There was no significant difference ($P>0.05$) in the levels of SOD,

CAT, GPx and MDA in the liver of rats treated with ginger only when compared to control.

Effect of treatment on histology

Figure 1 represents the histological results. Liver slides from the control group showed normal liver histoarchitecture; hepatocytes, sinusoids and the portal area. The slide of rats treated with CCL4 only showed histological distortions; fatty-impregnated vacuoles (steatosis). The histology of the liver of rats treated with ginger only and in combination with CCL_4 showed similar histology with the control.

Table 1: Liver function test across experimental groups

Grouping	AST (U/L)	ALT (U/L)	ALP (U/L)	TB (mg/dl)
Group A (Control)	69.000 ± 10.000	20.670 ± 4.910	34.390 ± 2.018	0.176 ± 0.024
Group B (Ginger)	81.330 ± 3.930	31.330 ± 5.364	47.310 ± 8.194	0.252 ± 0.025
Group C (CCL_4)	113.300 ± 5.239*	89.330 ± 7.796*	66.000 ± 2.309*	0.500 ± 0.058*
Group D (Ginger and CCL_4)	84.670 ± 4.333 #	32.670 ± 9.735 #	36.980 ± 5.072 #	0.202 ± 0.013 #

Data is represented as Mean ± SEM; * and # represent P<0.05 when compared with control and CCL_4 respectively.

Table 2: Antioxidant enzyme analysis across experimental groups

Grouping	SOD (U/g)	CAT (U/g)	GPx (U/g)	MDA (mol/g)
Group A (control)	0.503 ± 0.01	0.150 ± 0.006	1.287 ± 0.057	0.287 ± 0.032
Group B (Ginger only)	0.455 ± 0.031	0.134 ± 0.008	1.163 ± 0.099	0.218 ± 0.012
Group C (CCL_4 only)	0.257 ± 0.008*	0.083 ± 0.004*	0.532 ± 0.057*	0.802 ± 0.030*
Group D (Ginger and CCL_4)	0.530 ± 0.040 #	0.150 ± 0.007 #	1.325 ± 0.891 #	0.2685 ± 0.009 #

Data is represented as Mean ± SEM; * and # represent P<0.05 when compared with control and CCL_4 respectively.

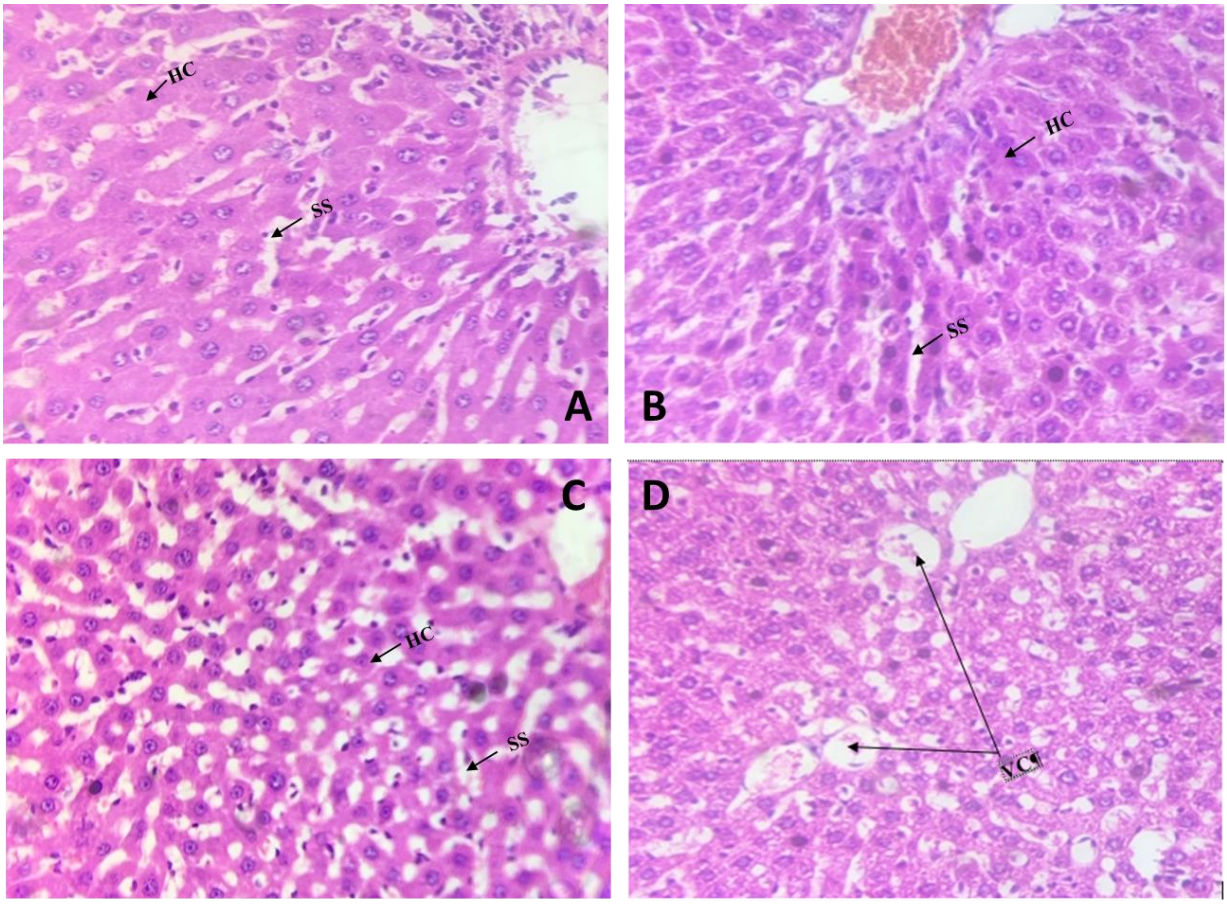

Figure 1: Histology of the liver across experimental groups (A) Control group showing hepatocytes (HC), sinusoids (SS). (B) Ginger-only group showing hepatocytes (HC), and sinusoids (SS). (C) CCL_4-only group showing severe multiple vacuolated hepatocytes (HC) and sinusoids (SS). (D) Ginger and CCL_4 group showing mild vacuolation (VC). H&E; 100x

Discussion

This study investigated the hepatoprotective activity of ginger in a CCl_4 model of hepatotoxicity. Reports indicate that the hepatoxic effect induced by CCl_4 is effected by two reactive and highly unstable free radicals, the trichloromethyl radical (CCl_3•) and the trichloromethyl peroxyl radical (Cl_3COO•) (Bekkouch *et al*., 2022). The formation of these two unstable free radicals serves as the origin of cellular damage via the induction of membrane lipid peroxidation. This is associated with the release of cytosolic and endoplasmic enzymes, indicating damage to liver structure and function (Malhi and Gores, 2008). The damage induced at the liver is accompanied by the elevation of serum liver markers such as AST, ALT, ALP and TB, as previously reported (Sabina *et al*., 2009). This reflects the presence of a rupture of the hepatic plasma membrane because these enzymes are primarily intracellular. AST, for example, is found in hepatocytes as two isoenzymes, one cytoplasmic, the other mitochondrial, and therefore, the presence of this enzyme in the extracellular medium signals the presence of damage in the liver cell (Wang *et al.,* 2011). On the other hand, exposure to hepatotoxic agents, which leads to liver parenchymal injury, also leads to an elevation of plasma bilirubin concentration (Darwish *et al.,* 2013). This can be explained by damage to the bile ducts or affection of the erythrocyte membrane by reactive species thus leading to hemolysis, and finally, the elevation of bilirubin levels (Awad *et al.,* 2018). The ability of ginger to protect these liver markers against CCl_4 indicates its potent hepatoprotective activity.

For a better understanding of the hepatoprotective effect of ginger, the liver antioxidant enzyme markers SOD, CAT and GPx were measured. The results showed a significant increase of the liver enzyme markers in the CCl_4-treated group when compared to the control, however, cotreatment of rats with ginger and CCl_4 attenuated the dysregulation of these enzymes by CCl_4. Also the increase in lipid peroxidation marker, MDA, highlighted the toxicity of CCl_4, although this was inhibited in the rats cotreated with ginger. These findings are comparable to those obtained by Oke *et al*. (2019), Hasan *et al*. (2016) and Abdel-Azeem *et al*. (2013) demonstrating that ginger extract mitigates CCl_4-induced liver toxicity. The ability to restore the liver enzyme markers could be attributed to the different bioactive compounds contained in ginger.

Administration of a hepatotoxic agent, whatever its nature, its dose, or its route of administration, for example, CCl_4, causes modification of the membrane permeability followed by tissue damage, cell necrosis, hepatic cell lysis, damage to the lysis of liver cells, damage to the bile ducts and/or loss of the functional integrity of the liver tissue architecture (Huang, 2009). The histological findings from this study such as fatty-impregnated vacuoles (steatosis) and hepatocyte degeneration in the liver of CCl_4-treated rats agree with previous studies demonstrating the susceptibility of the liver to toxins (Enogieru *et al*., 2015a; Enogieru *et al*., 2015b). The ability of ginger to prevent CCl_4-induced alterations to the liver indicates its hepatoprotective activity which could be attributed to previously reported bioactive compounds found in the plant (Enogieru and Momodu, 2022).

Put together, the findings from this study indicate that ginger could be an effective hepatoprotective agent against CCl_4-induced liver toxicity in adult Wistar rats. It is therefore recommended that further studies, aimed at investigating novel mechanisms of action and its possible application as an alternative treatment option for liver diseases, be carried out.

References

1. Abdel-Azeem AS, Hegazy AM, Ibrahim KS, Farrag A-RH, El-Sayed EM. (2013). Hepatoprotective, Antioxidant, and Ameliorative Effects of Ginger (*Zingiber officinale Roscoe*) and Vitamin E in Acetaminophen Treated Rats. Journal of Dietary Supplements. 10(3):195–209.

2. Awad A, Khalil SR, Farag MR, Nassan MA. (2018). Differential susceptibility of kidneys and livers to proliferative processes and transcriptional level of the genes encoding desmin, vimentin, connexin 43, and nestin in rats exposed to furan. Ecotoxicology and Environmental Safety. 162:235–244.

3. Bekkouch O, Dalli M, Harnafi M, Touiss I, Mokhtari I, Assri SE, Harnafi H, Choukri M, Ko S-J, Kim B, (2022). Ginger (Zingiber officinale Roscoe), Lemon (Citrus limon L.) Juices as Preventive Agents from Chronic Liver Damage Induced by CCl4: A Biochemical and Histological Study. Antioxidants. 11(2):390.

4. Darwish SF, El-Bakly WM, Arafa HM, El-Demerdash E. (2013). Targeting TNF-α and NF-κB Activation by Bee Venom: Role in Suppressing Adjuvant Induced Arthritis and Methotrexate Hepatotoxicity in Rats. Ashour HM, editor. PLoS ONE. 8(11): e79284.

5. Drury R, and Wallington E. (1980). Carleton's histological technique 5th ed. New York: Churchill Livingstone.

6. Enogieru AB, Charles YO, Omoruyi SI, Momodu OI. (2015a). Phyllanthus amarus: A hepatoprotective agent in acetaminophen induced liver toxicity in adult Wistar rats. SMU Med J. 2(1):150-165.

7. Enogieru AB, Charles YO, Omoruyi SI, Momodu OI, Ezeuko VC. (2015b). Stem bark extracts of Ficus exasperata protects the liver against paracetamol induced toxicity in Wistar Rats. Journal of Applied Sciences and Environmental Management. 19(1):155-159.

8. Enogieru AB, Omoruyi SI, Hiss DC, Ekpo OE. (2018). Potential antiparkinsonian agents derived from South African medicinal plants. Journal of herbal medicine. 13:1-7.

9. Enogieru AB, Momodu OI. (2021). African medicinal plants useful for cognition and memory: Therapeutic implications for alzheimer's disease. The Botanical Review. 87:107-134.

10. Enogieru AB, Momodu O. (2022). Neuroprotective effects of Zingiber officinale against lead-induced toxicity in Wistar rats. Nutrire. 48(1):2.

11. Enogieru AB, Omoruyi SI. (2022). Exploration of Aqueous Phyllanthus amarus Leaf Extract as a Protective Agent in Mercury Chloride-Exposed Wistar Rats: A Neurobehavioural Study. Journal of Applied Sciences and Environmental Management. 30; 26(4):629-637.

12. Grzanna R, Lindmark L, Frondoza CG. (2005). Ginger-An Herbal Medicinal Product with Broad Anti-Inflammatory Actions. Journal of Medicinal Food. 8(2):125–132.

13. Habsah M, Amran M, Mackeen MM, Lajis NH, Kikuzaki H, Nakatani N, Rahman AA, Ghafar null, Ali AM. (2000). Screening of Zingiberaceae extracts for antimicrobial and antioxidant activities. Journal of Ethnopharmacology. 72(3):403–410.

14. Hasan IH, El-Desouky MA, Abd-Elaziz GM, Hozayen WG. (2016). Protective effects of Zingiber officinale against carbon tetrachloride-induced liver fibrosis. International Journal of Pharmacy and Pharmaceutical Sciences. 8(3):377–381.

15. Huang Q, Xie Q, Shi C-C, Xiang X-G, Lin L-Y, Gong B-D, Zhao G-D, Wang H, Jia N-N. (2009). Expression of angiotensin-converting enzyme 2 in CCL4-induced rat liver fibrosis. International Journal of Molecular Medicine. 23(6):717–723.

16. Jain SK, Prakash V. (1995). Published by Indian Association for Angiosperm Taxonomy www.rheedea.in Zingiberaceae in India: Phytogeography and Endemism. Rheedea . 5(2):154–169.

17. Joy PP, Thomas JSM, Mathew S, Skaria BP. (1998). Medicinal Plants. Tropical Horticulture. 2:449–632.

18. Lunn RM, Mehta SS, Jahnke GD, Wang A, Mary Leigh Wolfe, Berridge BR. (2022). Cancer Hazard Evaluations for Contemporary Needs: Highlights from New National Toxicology Program Evaluations and Methodological Advancements. Journal of the National Cancer Institute. 114(11):1441–1448.

19. Malhi H, Gores GJ. (2008). Cellular and Molecular Mechanisms of Liver Injury. Gastroenterology. 134(6):1641–1654.

20. National Research Council. (2010). *Guide for the Care and Use of Laboratory Animals, 8th edition. National Academies Press*.

21. Oke GO, Abiodun AA, Imafidon CE, Monsi BF. (2019). Zingiber officinale (Roscoe) mitigates CCl4-induced liver histopathology and biochemical derangements through antioxidant, membrane-stabilizing and tissue-regenerating potentials. Toxicology Reports. 6:416–425.

22. Park EJ, Pezzuto JM. (2002). Botanicals in Cancer Chemoprevention. Cancer and Metastasis Reviews. 21(3/4):231–255.

23. Sabina E, Samuel J, RajappaRamya S, Patel S, Mandal N, Pranatharthiiharan P, Mishra PP, Rasool M. (2009). Hepatoprotective and antioxidant potential of Spirulina fusiformis on acetaminophen-induced hepatotoxicity in mice. International Journal of Integrative Biology. 6(1):1–5.

24. Sacchetti G, Maietti S, Muzzoli M, Scaglianti M, Manfredini S, Radice M, Bruni R. (2005). Comparative evaluation of 11 essential oils of different origin as functional antioxidants, antiradicals and antimicrobials in foods. Food Chemistry. 91(4):621–632.

25. Seifert WF, Bosma A, Brouwer A, Hendriks HFJ, Roholl PJM, van Leeuwen REW, van Thiel-De Ruiter GCF, Seifert-Bock I, Knook DL. (1994). Vitamin A deficiency potentiates carbon tetrachloride-induced liver fibrosis in rats. Hepatology. 19(1):193–201.

26. Vasala PA. Ginger. Peter K. V., Ed. (2004). Handbook of Herbs and Spices, Woodhead Publishing: Cambridge, UK.

27. Wang Y, Kirpich I, Liu Y, Ma Z, Barve S, McClain CJ, Feng W. (2011). Lactobacillus rhamnosus GG Treatment Potentiates Intestinal Hypoxia-Inducible Factor, Promotes Intestinal Integrity and Ameliorates Alcohol-Induced Liver Injury. The American Journal of Pathology. 179(6):2866–2875.