RIBOCEINE REGIMEN CIRCUMVENTS RESERPINE-INDUCED HEPATOTOXICITY VIA THE MODULATION OF KEY LIVER FUNCTION MARKERS IN ADULT MALE WISTAR RATS

ABSTRACT

Reserpine, an antipsychotic and antihypertensive medication, has been associated with liver damage and dysfunction. This study examined the potential hepatoprotective effect of a riboceine regimen against reserpine-induced hepatotoxicity in adult male Wistar rats. Twenty-five Adult male Wistar rats were randomly assigned to five groups: Control, Reserpine, Reserpine + Citalopram, Reserpine + Riboceine, and Reserpine + Citalopram + Riboceine. Liver function markers, including alanine transaminase (ALT), aspartate transaminase (AST), and alkaline phosphatase (ALP), were analyzed in serum samples to assess liver health. Additionally, a histopathological examination of liver tissue was performed to visualize any morphological changes. Serum ALT and AST levels significantly increased in rats given reserpine alone compared to the control group, indicating hepatocellular damage. In contrast, the Riboceine + Reserpine group exhibited a significant reduction in ALT and AST levels compared to the Reserpine group, suggesting a protective effect of riboceine against reserpine-induced hepatotoxicity. No significant difference was observed in the serum level of ALP across the experimental groups. Histopathological examination confirmed the attenuated liver injury in the Riboceine + Reserpine group, with a reduction in necrotic areas and inflammation compared to the Reserpine group. The results demonstrate that a riboceine regimen effectively circumvents reserpine-induced hepatotoxicity in adult male Wistar rats. The modulation of key liver function markers, along with histopathological evidence, supports the hepatoprotective role of riboceine in mitigating liver damage induced by reserpine. This study provides promising insights into the possible therapeutic application of riboceine as a hepatoprotective agent, which could be beneficial for patients undergoing treatment with reserpine or similar medications.

Keywords: Reserpine, Riboceine, Hepatotoxicity, Oxidative stress, Citalopram

1. Introduction

Depression is one of the most prevalent mental health disorders (1) affecting numerous individuals at some stage in their lives (2). Depression can manifest through a wide range of symptoms, from fatigue to suicidal thoughts. Its etiology can stem from internal or external factors such as genetics, stress, and drug abuse (3). On a molecular level, depression can disrupt synaptic plasticity in the brain and cause atrophic changes in the cortical and hippocampal regions (2). Research studies have suggested that a substantial decrease in monoamine neurotransmitters can trigger depression. Reserpine, an antihypertensive medication, has been used as a model to support the monoamine depletion hypothesis. Following reports of depression among multiple reserpine users, scientists found that it indeed led to a notable reduction in the production of monoamine neurotransmitters (4).

Reserpine, a climbing shrub native to India is an alkaloid derived from the plant, *Rauwolifia serpentine*. The clinical application of reserpine has been associated to manage hypertension, insanity, insomnia and schizophrenia (5). However, the use of reserpine as a drug is restricted because it has been associated with liver and other organ damage due to its ability to induce excess free radical production and oxidative stress (6).

Reserpine is known to act via irreversible blockade of VMAT-2 (vesicular monoamine transporter-2) and hence the inhibition of alpha-adrenergic neurotransmission pathway (5). The blockage of catecholamine pumps prevents the uptake of dopamine, norepinephrine, and serotonin into presynaptic storage vesicles.

Oxidative stress occurs from antioxidants and prooxidants imbalance in the body. Antioxidants, while typically protective, sometimes act as prooxidants under certain conditions. In aerobic environments, they produce superoxide radicals that dismutase to form H2O2, which, in turn, reacts with reduced metal ions and superoxide to generate toxic reactive oxygen species (ROS) (7).

The induction of oxidative stress results from radical species production and the antioxidant defense systems imbalance. This imbalance can lead to damage in cellular biomolecules, including lipids, proteins, and DNA (8). Reactive oxygen species (ROS) interact readily with all cellular macromolecules due to their reactivity. Specifically, Muriel and Gordillo (9) highlighted

that ROS can cleave phosphodiester bonds holding bases in RNA and DNA together, thereby disrupting the chain structure of these molecules. Excessive ROS production directly interacts with cellular biomolecules, such as DNA, lipids, and proteins, leading to modifications that may result in cell death (10).

Liver diseases represent a global health concern as the liver serves as the principal detoxification organ and plays important role in maintaining metabolic balance. The liver metabolizes various compounds that produce reactive oxygen radicals (11).

Oxidative stress disrupts redox balance, impacting liver function and influencing inflammatory pathways, contributing to various liver diseases. It is implicated in acute liver injury, the pathogenesis of prevalent infectious or metabolic chronic liver diseases, and in the progression of liver diseases to liver fibrosis, cirrhosis, and hepatocellular carcinoma (HCC) as discussed by Allameh et al (12).

Riboceine, a patented molecule, has been shown to effectively deliver cysteine molecules into cells, enabling them to produce optimal amounts of glutamate (13). It contains ribose and cysteine, which are naturally present in the human body. Riboceine aids cells in producing glutathione as needed with the active ingredient of riboceine being D-ribose-L-cysteine (14).

2. Material and Methods

2.1 Major Apparatus and Equipment

Plastic cages, syringe and needle, plain bottles, rat feed, sensitive weighing balance, cotton wool, dissecting set, measuring cylinder, flasks, test tubes, surgical gloves, scalpel blade, heparinized bottles, soap, surgery pins, oral cannula, bucket, dissecting board, sponge, mortar and pestle, blender, centrifuge machine, microscope slides, improved neubauer haemytometer, refrigerator and cover slips.

2.2 Experimental Animals and Care

Twenty-five (25) adult male rats with an average weight of 132 (± 10g) were used in the experiment. All rats were procured from the Animal Facility of Bright farm, Owode Ede, Osun state, Nigeria. The rats were bred in the animal house of the facility of Basic Medical Sciences, Osun State University, Osogbo, Osun State; under the light and dark cycle at room temperature

35□. Proper aeration was maintained by the use of wire gauze plastic cage. The Health Research Institute Ethics Committee (HREC) at Osun State University, Nigeria, approved the procedures employed in this study. During the study, rodent diet was obtained from Top feed Mills, Osogbo, Nigeria and clean drinking water was made available without restriction. Prior to administration, the rats were randomly placed in five groups and kept in separate cages and acclimatized to the experimental room 1 week before the start of experiments.

2.3 Experimental Design

Twenty-five adult male albino wistar rats were placed randomly into 5 groups designated as A, B, C, and D and E with 5 rats (n=5) in each group. The groupings and dosage of treatments are as follows;

- Group A - Control group (physiological saline and feed).
- Group B - Induced with 50mg/kg reserpine only.
- Group C - Induced with 50mg/kg reserpine and co-treated with citalopram (40mg/kg).
- Group D - Induced with reserpine (50mg/kg) and co-treated with riboceine (30mg/kg).
- Group E - Induced with reserpine and co-treated with citalopram(40mg/kg) and riboceine (30mg/kg).

All animals except those in control group were induced with reserpine. Animals in group C were administered Citalopram and Reserpine and animals in group D and E were administered Riboceine and Reserpine after one week of acclimatization.

2.4 Drugs and Reagents

Reserpine (Calbiochem – 506238) and citalopram (Calbiochem – 506130) were purchased from the United States. Riboceine (Cellgevity – 703327642433) was purchased locally in Nigeria. Distilled water, formaldehyde, normal saline, and every other chemical, materials, drugs and reagents used were procured locally and to analytical grade.

2.5 Ethical Approval

All experimental procedures adhered to the requirement set by the Health Research Ethics Committee (College of Health Sciences, Osun State University, Osogbo, Nigeria) complying with

the guidelines of the National Institute of Health guide for the care and use of Laboratory Animals. The research was conducted in the Animal House facility of Osun State University in Osogbo, Osun State, Nigeria.

2.6 Drug Preparation and Administration

The volume administered was calculated using the stock solutions and the average body weight (ABW). In order to obtain the volume gain = Average body mass/1000 ×dosage (mg/kg) / stock solution. With the average body weight at 132; reserpine, citalopram and riboceine was administered.

Stock solution of Reserpine was prepared by dissolving 54.5mg of the drug in 10ml of distilled water (i.e 1ml of solution contains 5.45mg of drug).

Stock solution of Citalopram was prepared by dissolving 245.6mg in 10ml of distilled water to give 24.56mg/ml.

Stock solution of Riboceine was prepared by dissolving 630mg in 10ml of distilled water to give 63 mg/ml.

Volume of Reserpine administered =132/1000×50/545 = 0.1ml

Volume of Citalopram administered = 132/1000 × 40/2456 = 0.1ml

Volume of Riboceine administered = 132/1000 × 30/630 =0.1ml

Volume of Reserpine was 0.2 ml for group E.

Volume of citalopram was 0.2ml for group E.

Volume of Ribociene was 0.2ml for group E.

2.7 Animal Sacrifice and Tissue Processing

Rats were euthanized with intramuscular injection of ketamine (20mg/kg) for histological assessment 12 hours after the final treatments. Blood was withdrawn from the left ventricle and transferred into heparinized bottles. Transcardial perfusion was then performed beginning with a 50 ml flush of 0.1 M PBS (pH 7.4) after which 500 ml of 4% para-formaldehyde (PFA) was infused via cardiac puncture. The liver was then excised and fixed in 4% PFA for 24 hours then stored in

30% sucrose at 4 □. Histological demonstration was conducted on sections embedded in paraffin wax, which were stained with Hematoxylin and Eosin for general cytoarchitectural demonstration.

2.8 Routine Histology

2.8.1 Hematoxylin and Eosin Staining Procedure

The sections were first deparaffinized in two changes of xylene, each lasting three minutes, followed by rehydration in two changes od descending grades of alcohol, including absolute I, absolute II, 90%, 70%, and 50% ethanol, for two minutes each. After rinsing in distilled water for three minutes, the sections were stained with ion hematoxylin for 10 to 15 minutes. Excess stain was then washed away under running tap water for five minutes, and the sections were differentiated in 1% acid alcohol for one minute. Following this, the sections were counterstained with eosin for two minutes. Finally, they were dehydrated through ascending grades of alcohol, each for two minutes, cleared in two changes of xylene, and mounted in a synthetic resin medium (D.P.X).

2.9 Biochemical Assay

2.9.1 Estimation of Liver function Markers

Blood samples collected were centrifuged at 3000 RPM for 15 minutes to separate the serum. An automated biochemical analyzer was then used to measure ALP, AST, and ALT levels in the serum. The manufacturer's instructions for sample loading and analysis were strictly followed and the results were recorded in appropriate units for each enzyme.

2.10 Photomicrography

The sections were observed using a Leica DM750 research microscope equipped with a Leica ICCS50 digital camera. Photomicrographs of the tissue sections were captured at multiple magnifications.

2.11 Statistical Analysis

Biochemical examination results were quantitatively analyzed with GraphPad Prism (version 8) software. One-way Anova and Tukey’s multiple comparison test were applied, setting significance at $p < 0.05$.

3. Result

3.1 Effect of Riboceine on serum Aspartate Transferase level in Reserpine treated Rats

As displayed in figure 1, serum AST level increased significantly ($P<0.05$) in rats that received reserpine only (Dep) with a mean value of 69.40±2.71as well as rats treated with reserpine and citalopram (Dep+AntiDep) with a mean value of 70.12±1.33 when compared with the control group (51.60±2.11); Supplementation with riboceine significantly ($P>0.05$) modulated the serum AST level of rats in group D (69.40±2.71) and E (69.40±2.71) which was significant when compared to rats in groups B and C.

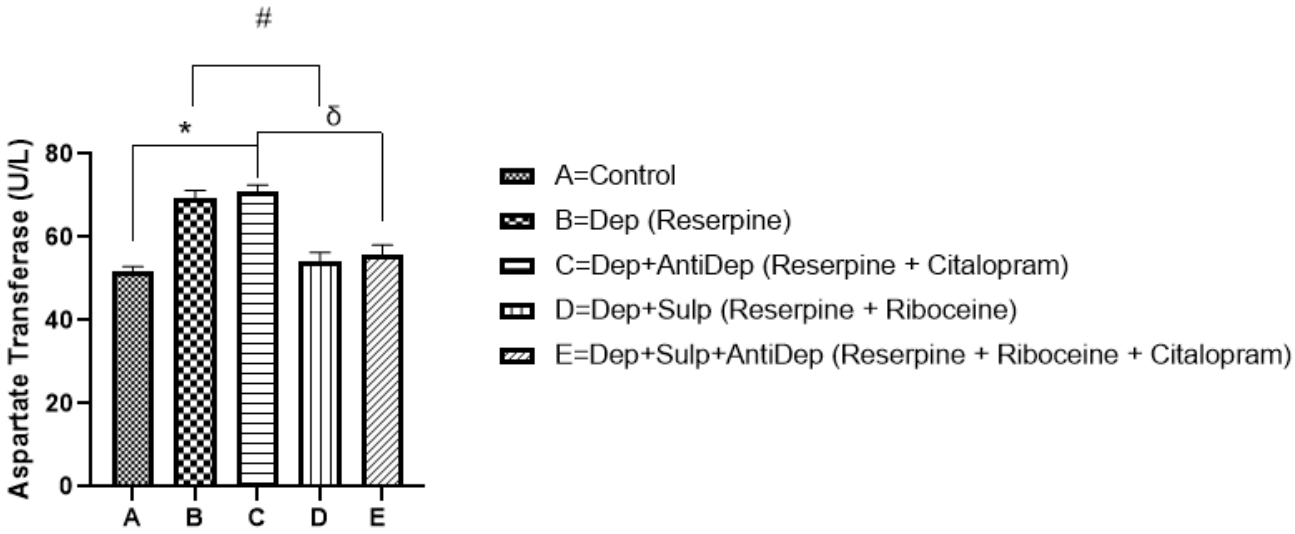

Figure 1: AST level across all groups.*****represent significant difference when compared with group A**;** $^{\#}$ represent significant difference when compared with group D; $^{\delta}$ represent significant difference when compared with group E.

3.2 Serum Alanine Transaminase Concentration was Modulated by Riboceine Regimen

In this study, serum ALT profile was observed to increase significantly in rats administered with reserpine only (group B) with a mean value of 26.61±2.34 when compared with the control (18.20±4.21) group and rats treated with reserpine and riboceine (Dep+Sulp group; 18.10±1.71). Concomitant treatment with reserpine, citalopram, and riboceine (group E; 19.31±3.25) also

elicited a significant reduction in the level of serum ALT when compared to the reserpine only group (Figure 2).

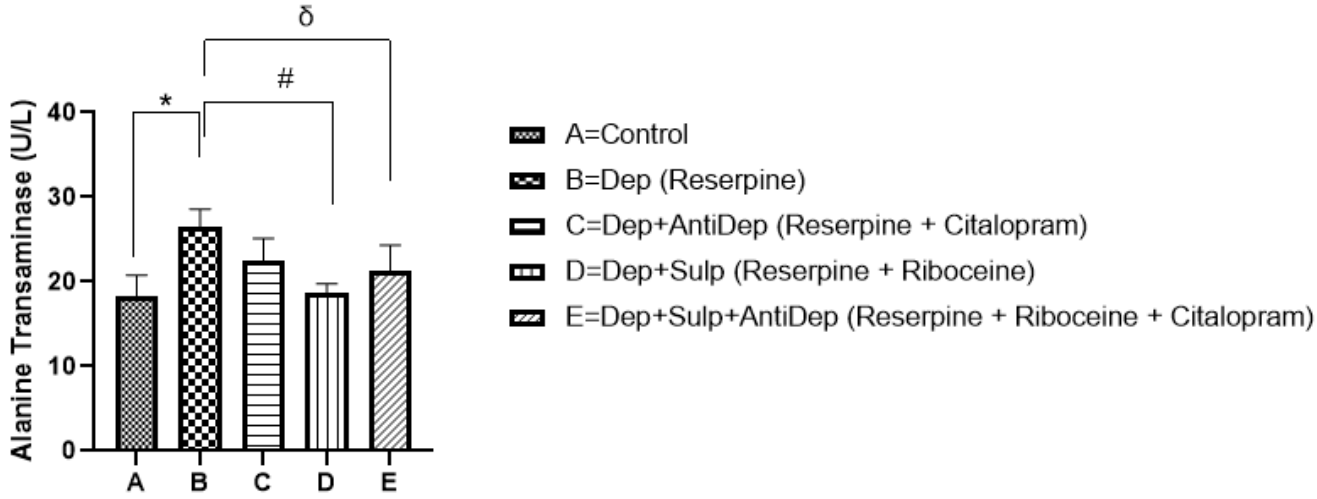

Figure 2: ALT Levels across the experimental groups. ***** represents significant difference when compared with group A**;** # represent significant difference when compared with group D; δ represent significant difference when compared with group E.

3.3 Effects of Reserpine, Citalopram, and Riboceine on Serum Alkaline Phosphate Concentration

In figure 3, no significant difference was observed in the serum alkaline phosphate level across all the experimental groups.

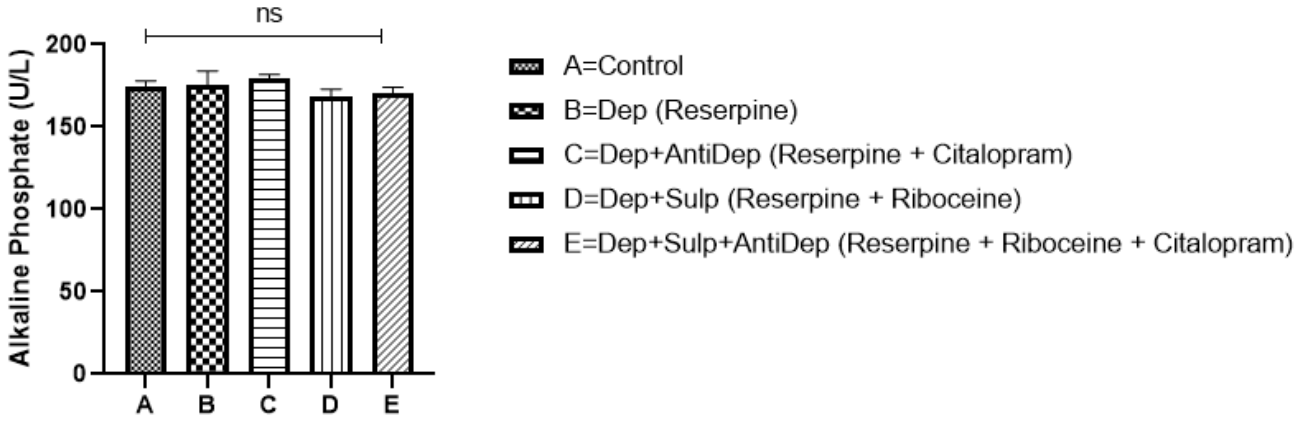

Figure 3: ALP Level across the experimental groups**.***represent significant difference when compared with group A**;** # represent significant difference when compared with group D; δ represent significant difference when compared with group E.

3.4 Histological Evaluation of the Liver

As shown in fig 4, reserpine only treated rats (group B) showed saltered histo-morphological presentation (red arrows) when compared to group A micrograph. This alteration is characterized by necrotic hepatocytes, poorly stained hepatocytes and ruptures in the walls of the hepatic vessels. Furthermore, rats treated with citalopram and reserpine (group C) showed mild pathological changes however the general presentation was similar to the group. Riboceine treated rats (groups D and E) presented with a normal liver microarchitecture consistent with the control group.

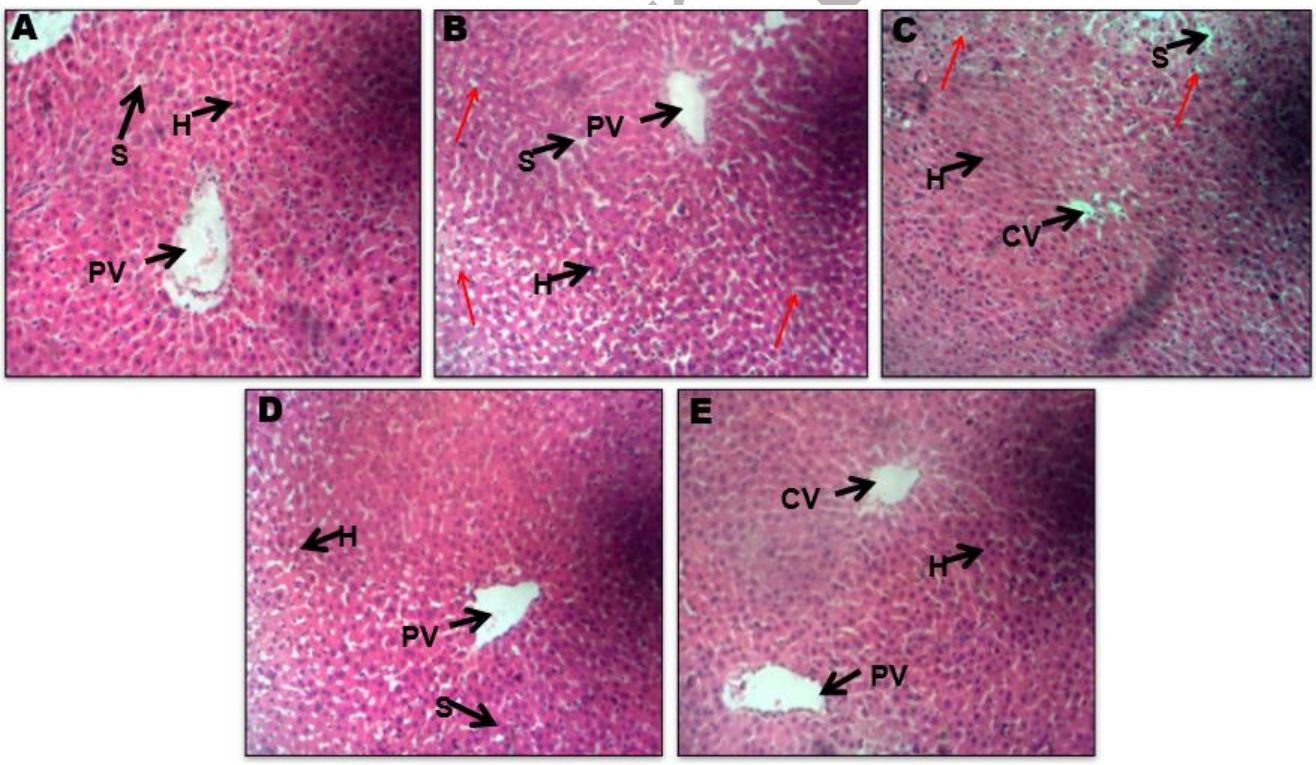

Figure 4: Photomicrographs of the liver general micromorphological presentation across the study groups (A-E). Hematoxylin and Eosin stain (x100). The hepatocytes (H), Portal vein (PV), central vein (CV) and stroma (S) are well outlined across the micrographs.

4. Discussion

The objective of this study was to examine the potential protective effect of a riboceine regimen against reserpine-induced hepatotoxicity in adult male Wistar rats. Reserpine, an antipsychotic and antihypertensive medication, has been associated with hepatotoxic effects, leading to liver damage and dysfunction as discussed by Weir (15). The liver plays a crucial role in metabolism, detoxification, and protein synthesis, making it susceptible to drug-induced injury (16).

The findings of this study showed that the administration of a riboceine regimen effectively mitigated the hepatotoxic effects of reserpine, as evidenced by the modulation of key liver function markers in the experimental animals. The markers including alanine transaminase (ALT), aspartate transaminase (AST), and alkaline phosphatase (ALP) which are widely used in clinical practice to assess liver health and detect liver injury (17).

In this study, the rats treated with reserpine alone exhibited a significant increase in ALT and AST levels compared to the control group, confirming the hepatotoxic effect of reserpine. Elevated serum levels of ALT and AST are indicative of hepatocellular damage (ref) reserpine hepatotoxicity observed in this study can be attributed to its ability of inducing excess free radical production and oxidative stress which are consistent with the report of Khushboo et al., (6)

The results from this study showed that citalopram treatment had no significant effect against reserpine induced hepatotoxicity. This may be as a result of its mechanism of action which purely relates to the nervous system (18). Conversely, rats that received the riboceine regimen along with reserpine demonstrated a substantial attenuation of the reserpine-induced hepatotoxicity, as shown by the marked reduction in ALT and AST levels. This observation is consistent with finding by Mega Obukohwo et al (5) which suggests that riboceine exerts a hepatoprotective effect preventing or reducing liver cell damage induced by reserpine.

Riboceine is known to enhance the production of glutathione (GSH), a crucial intracellular antioxidant that plays a significant role in neutralizing free radicals and protecting cells from oxidative stress (19). Reserpine-induced hepatotoxicity is often associated with increased oxidative stress (20), and the enhanced GSH production by riboceine (21) may counteract this damaging effect, leading to a reduction in liver cell injury (22). Also, Hepatotoxicity is often

accompanied by inflammation in the liver tissue (23). Riboceine has been reported to possess anti-inflammatory properties (5), which could contribute to its protective effects against reserpine-induced liver injury.

Furthermore, riboceine may modulate specific cell signaling pathways involved in liver injury and repair, leading to a restoration of normal liver function (5). The exact molecular mechanisms underlying the hepatoprotective action of riboceine remain to be fully elucidated, but several potential mechanisms could be responsible for its beneficial effects which include significant reduction in malondialdehyde (MDA) and C-reactive protein levels in liver cells thereby protecting them from oxidative and inflammatory injury (24).

Histological examination of photomicrographs of the different treatment groups corresponds with the liver function test findings earlier discussed. The rats treated with reserpine had very poor hepatic morphology characterized by necrotic plaques and ruptured hepatic vessels (20). Riboceine treated rats however, appeared normal and showed very similar morphology with the control group. This further goes to affirm the beneficial role of riboceine in circumventing hepatic perturbations induced by reserpine.

The observed protective effect of riboceine in this study has important implications for clinical applications. As a potential hepatoprotective agent, riboceine may hold promise in preventing or reducing drug-induced liver injury caused by reserpine and possibly other hepatotoxic substances. However, further studies are needed to validate these results in human subjects and explore the optimal dosing and treatment duration of riboceine for hepatoprotection.

In conclusion, the findings of this study indicate that a riboceine regimen effectively circumvents reserpine-induced hepatotoxicity in adult male Wistar rats. The modulation of key liver function markers, including ALT, AST, and ALP, supports the potential hepatoprotective role of riboceine. This research opens avenues for future investigations into riboceine as a therapeutic strategy to safeguard liver health and mitigate drug-induced liver injury in clinical settings. Nonetheless, caution should be exercised in extrapolating these findings to human subjects until further clinical evidence is available.

Authors' Contribution

Study concept and design: A.O.S.

Acquisition of data: J.O.F., A.O.O.

Analysis and interpretation of data: A.0.S., B.I.O., O.A.O.

Drafting of the manuscript: I.0.B., A.O.O.

Critical revision of the manuscript: A.O.S., I.O.B., B.I.O., O.A.O.

Statistical analysis: J.O.F.

Ethics

It is declared that all ethical considerations were taken into account in the preparation of the submitted manuscript.

Acknowledgement

Special thanks to the staffs of Laboratory Unit, Department of Anatomy, Osun State University for their technical supports in the course of carrying out this work

Conflict of Interest

The authors declare that they have no conflict of interest.

References

1. Schimelpfening N. 7 Facts You Should Know About Depression. Verywell Mind. 2023 Jun 16. Available from: https://www.verywellmind.com/depression-facts-you-should-know-1067617

2. Ren F, Guo R. Synaptic Microenvironment in Depressive Disorder: Insights from Synaptic Plasticity. Neuropsychiatric Disease and Treatment. 2021;17:157-165. doi: 10.2147/ndt.s268012.

3. Orsolini L, Latini R, Pompili M, Serafini G, Volpe U, Vellante F, et al. Understanding the Complex of Suicide in Depression: from Research to Clinics. Psychiatry Investigation. 2020 Mar 25;17(3):207-221. doi: 10.30773/pi.2019.0171.

4. Govindarajulu M, Shankar T, Patel S, Fabbrini M, Manohar A, Ramesh S, et al. Reserpine-Induced Depression and Other Neurotoxicity: A Monoaminergic Hypothesis. In: Medicinal Herbs and Fungi: Neurotoxicity vs. Neuroprotection. 2021. p. 293-313.

5. Mega Obukohwo O, Faith FY, Peggy Ejiro O, Onyebuchi MM, Ajayi AF. Medicinal applications of d-ribose l-cysteine in neuro-endocrinopathy, reproductive dysfunction and cardio-metabolic syndrome: A review of evidence in animal investigations. European Journal of Medicinal Chemistry Reports. 2024 Aug;11:100153. doi: 10.1016/j.ejmcr.2024.100153.

6. Khushboo, Gupta VK, Sharma B. Hepatoprotective Effect of Ethanolic Extract of Garlic Against Reserpine Induced Toxicity in Wistar Rats. Indian Journal of Clinical Biochemistry. 2022 Jun 14;38(2):251-261. doi: 10.1007/s12291-022-01045-9.

7. Sotler R. Prooxidant Activities of Antioxidants and Their Impact on Health. Acta Clinica Croatica. 2019;58. doi: 10.20471/acc.2019.58.04.20.

8. Juan CA, Pérez de la Lastra JM, Plou FJ, Pérez-Lebeña E. The Chemistry of Reactive Oxygen Species (ROS) Revisited: Outlining Their Role in Biological Macromolecules (DNA, Lipids and Proteins) and Induced Pathologies. International Journal of Molecular Sciences. 2021 Apr 28;22(9):4642. doi: 10.3390/ijms22094642.

9. Muriel P, Gordillo KR. Role of Oxidative Stress in Liver Health and Disease. Oxidative Medicine and Cellular Longevity. 2016;2016:1-2. doi: 10.1155/2016/9037051.

10. De La Rosa LC, Goicoechea L, Torres S, García-Ruiz C, Fernández-Checa JC. Role of Oxidative Stress in Liver Disorders. Livers. 2022 Oct 14. doi: 10.3390/livers2040023.

11. Casas-Grajales S, Muriel P. Antioxidants in liver health. World Journal of Gastrointestinal Pharmacology and Therapeutics. 2015;6(3):59.

12. Allameh A, Niayesh□Mehr R, Aliarab A, Barbàra M, Pantopoulos K. Oxidative Stress in Liver Pathophysiology and Disease. Antioxidants. 2023 Aug 22; doi: 10.3390/antiox12091653

13. Abayomi TA, Tokunbo OS, Ajayi M, Abayomi OA, Ofusori DA. Riboceine regimen attenuates ethanol-induced neuronal damage in the cerebellum of adult male Wistar rats. Iranian Journal of Toxicology. 2021;15(4):249-256.

14. Gee D. What is RiboCeine Technology and The Benefits? MyDiet234. 2018 Oct 11 [cited 2024 APR 12]. Available from: https://diet234.com/riboceine-technology/

15. Weir MR. Reserpine: A New Consideration of an Old Drug for Refractory Hypertension. American Journal of Hypertension. 2020 Apr 18;33(8):708-710. doi: 10.1093/ajh/hpaa069.

16. Alamri ZZ. The role of liver in metabolism: an updated review with physiological emphasis. International Journal of Basic & Clinical Pharmacology. 2018 Oct 23;7(11):2271. doi: 10.18203/2319-2003.ijbcp20184211

17. Todorovic T, Vujanovic D. The influence of magnesium on the activity of some enzymes (AST, ALT, ALP) and lead content in some tissues. Magnesium Research. 2002;15(3-4):173-177.

18. Shoar NS, Fariba KA, Padhy RK. Citalopram. StatPearls - NCBI Bookshelf. 2023 Nov 7 [cited 2024 APR 25]. Available from: https://www.ncbi.nlm.nih.gov/books/NBK482222/

19. Ukwenya VO, Alese MO, Ogunlade B, Folorunso IM, Omotuyi OI. Anacardium occidentale leaves extract and riboceine mitigate hyperglycemia through anti-oxidative effects and modulation of some selected genes associated with diabetes. Journal of Diabetes Metabolic Disorders. 2022 Dec 8. doi: 10.1007/s40200-022-01165-2.

20. Liu SB, Zhao R, Liu X, Guo H, Tian Z, Zhang N, et al. Attenuation of Reserpine-Induced Pain/Depression Dyad by Gentiopicroside Through Downregulation of GluN2B Receptors in the Amygdala of Mice. Neuromolecular Medicine. 2014 Mar 1. doi: 10.1007/s12017-013-8280-8.

21. Amole O, Akinyede A, Adewale M. Effects of riboceine (Cellgevity) on ulcer models in rats. 2020 Jun 27 [cited 2024 APR 17]. Available from: http://www.scigreen.com/index.php/JBPT/article/view/69

22. Vairetti M, Di Pasqua LG, Cagna M, Richelmi P, Ferrigno A, Berardo C. Changes in Glutathione Content in Liver Diseases: An Update. Antioxidants. 2021 Feb 28;10(3):364. doi: 10.3390/antiox10030364.

23. Bansal L, Dhiman A. Chronicity of hepatotoxicity: A Review. EAS J Med Surg. 2022;4:6-14.

24. Thomas AB. Modulatory Role of Riboceine and Vitamin C Supplementation on Altered Oxidative Stress Status of Alloxan-induced Diabetic Wistar Rats. Bioscience Biotechnology Research Communications. 2020 Dec 25;13(4):2130-2138. doi: 10.21786/bbrc/13.4/75.