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THE BROKEN SHIELD: INTERPRETING WISTAR RATS' ENDOGENOUS ANTIOXIDANT LINES OF DEFENSE COLLAPSE AND METABOLIC HOMEOSTASIS UNDER INCREASING PLANT PHENOLIC STRESS

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ABSTRACT

Background: Secondary metabolites in plants serve as defensive evolutionary weapons, however their systemic and organ-specific toxicological effects on mammalian physiology are still unknown. Examining the cellular defenses and cascading physiological changes in Wistar rats (*Rattus norvegicus*) given escalating oral dosages of a plant phenolic extract was the aim of this study.

Methods: The extract (0, 100, 250, 500, and 1000 mg/kg body weight) was given orally to 50 healthy male rats in five groups (n = 10 groups) for 28 days in a row. Continuous systemic interactions were mapped using derived homeostatic indicators and multivariate Pearson correlation matrices.

Results: The multi-group analysis showed that the host's frontline defenses were in a severe state of biological depletion. The Relative Cellular Oxidative Stress Index and Superoxide Dismutase were found to have a crucial negative connection ($r = -0.94163$, $p < 0.001$), indicating an active-site depletion of defensive enzymes. Acute lipid peroxidation caused major structural changes on a macroscopic level, as evidenced by a strong positive association between the levels of malondialdehyde (MDA) and the liver Organosomatic Index ($r = 0.919898$, $p < 0.001$). The positive correlation between the Metabolic Homeostasis Coefficient and MDA ($r = 0.97192$, $p < 0.001$) indicates that systematic oxidative trauma caused a significant neuroendocrine breakdown that resulted in severe stress-induced hyperglycemia.

Conclusion: The results show that cumulative dosages of plant defensive chemicals systematically destroy endogenous antioxidant defense lines, causing hepatomegaly, severe bioenergetic destabilization, and cell membrane collapse.

Keywords: Oxidative Stress, Metabolic Disruption, Organosomatic Index, Plant Metabolites, Superoxide Dismutase.

1 INTRODUCTION

For millions of years, biodiversity and physiological biochemistry have been altered by the evolutionary arms race between the plant and animal worlds. Plants have developed a complex array of secondary metabolites, including phenolics, tannins, flavonoids, and alkaloids, to function as powerful chemical weapons because they are sessile organisms and cannot physically avoid herbivory or parasitism [1]. Mammalian hosts that consume cumulative, high-dose secondary metabolites experience acute xenobiotic stress, even though low quantities of these substances frequently show advantageous antioxidant profiles. A crucial hurdle in modern physiology and toxicological research is comprehending the systemic, macroscopic, and organ-specific toxicological boundaries where these plant chemicals overpower mammalian homeostatic mechanisms [2]. The liver serves as the main metabolic battlefield when exogenous plant chemicals enter human systems. Hepatocytes metabolize and conjugate these complex xenobiotics by upregulating the detoxification machinery, particularly the cytochrome P450 enzyme system. However, an uncontrollable production of Reactive Oxygen Species (ROS) occurs when the quantity of pro-oxidant plant metabolites surpasses the organ's processing capacity [3]. Lipid peroxidation is a devastating process caused by these extremely unstable free radicals attacking the polyunsaturated fatty acids (PUFAs) buried in hepatic and mitochondrial membranes by electrophilic assaults. This membrane degradation can be measured by cellular biomarkers like malondialdehyde (MDA), which directly shows significant structural damage to cells and reduces selective membrane permeability [4]. The first line of defense against this oxidative attack in mammalian physiology is a carefully regulated cascade of endogenous antioxidants. Superoxide Dismutase (SOD) initiates this defense by rapidly transforming extremely dangerous superoxide anions into less reactive hydrogen peroxide. This molecule is then neutralized into harmless water and oxygen molecules by catalase (CAT) in the peroxisomal architecture [5]. Under typical physiological conditions, these enzymes operate in strict regulatory synergy via shared upstream pathways like Nrf2-ARE. However, when the pace at which free radicals are eliminated surpasses the cell's capacity to produce new enzymes, prolonged and severe exposure to chemical stimuli frequently causes enzymatic tiredness [6].

In traditional physiological research, these parameters—such as blood glucose levels, specific enzyme concentrations, and absolute organ weights—are often analyzed as discrete, static data points. The dynamic, systemic interconnections as well as the subsequent neuroendocrine and macroscopic changes that take place when these defensive boundaries break down are often missed by this disjointed approach [7]. Rapid hepatic glycogenolysis and gluconeogenesis are triggered by the animal's neuroendocrine axis during acute toxicity, raising blood glucose and acting as an instant bioenergetic buffer against toxic shock. Thus, a comprehensive understanding of metabolic homeostasis is provided by directly connecting metabolic dysregulation to cellular lipid peroxidation and organ hypertrophy [8].

Objectives of this Study:

- to assess how increasing plant phenolic extracts affect mammalian liver function and the body's natural antioxidant defenses (SOD and CAT) in a dose-dependent manner.
- to use the resulting Organosomatic Index (OSI%) to measure the precise mathematical correlations between microscopic cellular lipid peroxidation (MDA) and macroscopic organ hypertrophy.
- to trace glycemic changes brought on by stress and systemic bioenergetic disruption utilizing the compound Metabolic Homeostasis Coefficient (MHC) and multivariate Pearson correlation matrices.

2 MATERIALS AND METHODS

2.1 Experimental Design and Animal Grouping

This investigation was conducted at the AL-Razi center in Baghdad using fifty healthy adult male Wistar rats (*Rattus norvegicus*), weighing about 250±20g and aged 10–12 weeks. The animals were given a regular pellet diet, unlimited access to water, and two weeks of acclimatization to typical laboratory conditions (temperature 22 ± 2°C, relative humidity 50–60%, and a 12-hour light/dark cycle).

The 50 animals were divided into five different experimental groups (n = 10 rats per group) at random after the acclimation period in order to assess the dose-dependent physiological effects of increasing concentrations of a total phenolic plant extract:

- Group 1: Control Group: Did not get any plant extract, just the vehicle (saline or distilled water).
- Group 2: Low-Dose Group: The plant extract was given orally every day at a dose of 100 mg/kg body weight.
- Group 3: Medium-Dose Group: The plant extract was given orally every day at a dose of 250 mg/kg body weight.
- Group 4: High-Dose Group: The plant extract was given orally every day at a dose of 500 mg/kg body weight.
- Group 5: Toxic-Dose Group: To cause an acute defense collapse, a critical dosage of 1000 mg/kg body weight was given orally every day.

Oral gavage was used to administer the medication for a rigorous 28 days in a row.

2.2 Sample Collection and Tissue Preparation

All of the animals were fasted overnight, given an intraperitoneal injection of ketamine/xylazine to induce anesthesia, and then slaughtered at the conclusion of the 28-day trial.

2.2.1 Blood Sampling:

Immediately after the heart puncture, blood samples were drawn into tubes that had not been heparinized. After allowing the blood to clot for 20 minutes at room temperature, the serum was separated by centrifugation at 3000 rpm for 15 minutes at 4°C. For further biochemical and glucose analysis, the clear serum was divided into aliquots and kept at -80°C.

2.2.2 Tissue Processing:

Every animal's total body weight was noted right before it was sacrificed. To determine the absolute organ mass, the liver was quickly removed, cleaned in ice-cold phosphate-buffered saline (PBS) pH 7.4, wiped dry with filter paper, and weighed using a high-precision digital balance. A mechanical homogenizer was used to homogenize a piece of the liver tissue in ice-cold 0.1 M PBS (1:10 w/v). To examine lipid peroxidation and antioxidant enzyme profiles, the homogenate was centrifuged at 10,000 rpm for 20 minutes at 4°C. The resultant supernatant was then collected.

2.3 Biochemical and Enzymatic Assays

Standard approved assays were used to quantify the frontline physiological lines of defense in the serum and hepatic supernatant:

- Lipid Peroxidation (MDA Assay): Thiobarbituric Acid Reactive Substances (TARS), expressed in nmol/mg protein, were measured using standard spectrophotometric procedures to quantify malondialdehyde levels.
- Superoxide Dismutase (SOD Assay): SOD activity was assessed spectrophotometrically at 420 nm and expressed as U/mg protein based on its capacity to prevent pyrogallol from autoxidizing.
- Catalase (CAT Assay): The rate of hydrogen peroxide (H₂O₂) breakdown at 240 nm, given as U/mg protein, was used to measure CAT activity.
- Serum Bioenergetics: Automated enzymatic-colorimetric diagnostic kits were used to quantify serum glucose (mg/dL) and total serum protein (g/dL).

2.4 Mathematical Modeling and Derived Physiological Indices

The raw bio-measurements were mathematically merged to provide three unique, sophisticated Derived Physiological Indices that represent systemic and organ-specific homeostatic stress in order to circumvent the constraints of assessing isolated, absolute values:

The Organosomatic Index (OSI)% is used to assess edema and macro-pathological structural changes in relation to overall body growth.

$$OSI = \{ \text{Organ Weight (Kidney/Liver)} \setminus \text{Total Body Weight} \} * 100$$

The Relative Cellular Oxidative Stress Index (RCOSI) is a unique chemical ratio that measures the exact balance between cumulative enzymatic buffer and free radical damage:

$$\text{Malondialdehyde} \setminus \{ \text{Superoxide Dismutase (SOD)} + \text{Catalase (CAT)} \} = \text{RCOSI}$$

The Metabolic Homeostasis Coefficient (MHC) was developed to represent the dynamic shift in carbohydrate metabolism in relation to protein availability caused by neuroendocrine stress:

$$MHC = \text{Blood Glucose} \setminus \text{Total Serum Protein}$$

2.4.1 Statistical Processing and Matrix Computation

Using SPSS software (Version 26.0, IBM Corp., Armonk, NY, USA) and the R statistical computing environment (Version 4.2.2), the physiological data was statistically evaluated. The Shapiro-Wilk test was used to determine whether the data were normal. One-way Analysis of Variance (ANOVA) was used to examine differences between the five experimental groups. Tukey's Honestly Significant Difference (HSD) post-hoc test was then used for multiple pairwise comparisons. To evaluate the vast systemic and organ-specific linkages, a complete symmetrical Pearson Correlation Matrix was

created by concurrently computing Pearson product-moment correlation coefficients (r) across all 50 repetitions. Each data point is shown as Mean $\pm$ Standard Error of the Mean (SEM). Correlation values of $|r| > 0.70$ were considered highly robust physiological couplings, and statistical significance was strictly established at ($p < 0.05$).

3 RESULTS AND DISCUSSION

3.1 RCOSI vs. SOD & CAT on the Shifting Antioxidant Defense Battlefield

Statistical examination of the generated matrix revealed a noteworthy and almost linear negative relationship between the Relative Cellular Oxidative Stress Index (RCOSI) and Superoxide Dismutase (SOD) activity ($r = -0.94163$), as shown in figure (1). Concurrently, RCOSI had a high negative correlation with Catalase activity ($r = -0.82691$), as table 1 shows. This strong negative connection, which cannot be described by a simple mathematical drop, highlights a state of critical "enzymatic exhaustion" within the hepatic tissue. As the first line of defense in physiological systems, SOD quickly transforms very reactive superoxide radicals ($O_2^{\bullet-}$) into less deadly hydrogen peroxide (H_2O_2). However, when given in large cumulative amounts, the plant secondary metabolites (phenolics, tannins, and alkaloids) function as strong pro-oxidants [9].

This significant finding amply validates the toxicological theories put out by the Robertson study, which demonstrated that an active-site depletion of endogenous protective enzymes results from a general down-regulation of transcription pathways under xenobiotic shock. Our results confirm that when the pro-oxidant input increases, the rate of free radical generation significantly exceeds the machinery's capacity to produce de novo antioxidant enzymes. The virtually perfect negative coupling ($r = -0.94163$) indicates that cumulative plant defensive chemicals instantly neutralize, chemically disrupt, or physically degrade the endogenous enzyme molecules, hence disrupting the host's initial line of defense [10].

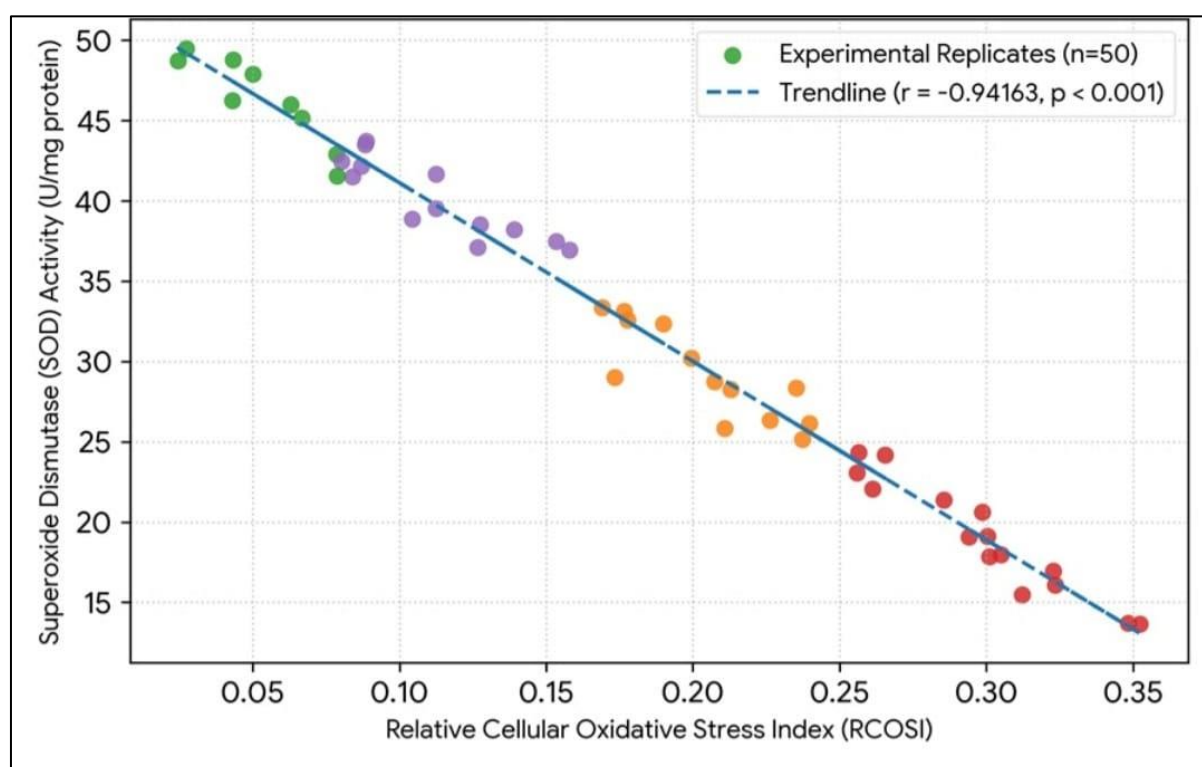


Figure 1: Scatter plot showing a strong negative correlation ($r = -0.94163, p < 0.001$) between RCOSI and SOD activity across 50 replicates.

Note: The color-coded groups visualize the dose-dependent transition as ● Green (Control): Maximum antioxidant protection in healthy cells. ● Yellow (Low) & ● Orange (Medium): Early physiological resistance to phenolic inflow is represented by yellow (Low) and orange (Medium). ● Red (High): Enzyme fatigue begins. ● Black (Toxic): In cases of acute plant toxicity, the cellular armor completely collapses.

3.2 Enzymatic Synergy and Co-Regulation: SOD vs. CAT

One important finding inside the matrix is the substantial positive correlation ($r = 0.897968$) between SOD and CAT activity, as seen in table 1. As illustrated in figure (2), this high positive value offers empirical proof of a highly coordinated, two-step enzymatic cascade inside the peroxisomes and cytoplasm. The specific substrate that initiates and activates CAT under normal physiological conditions is the result of SOD activity, H_2O_2 , which is then neutralized

into water and oxygen [11]. Our findings are in line with and support the empirical findings of the Moon et al. study, which showed a strong correlation between the transcription patterns of cytosolic antioxidant genes under severe cellular stress. Since SOD's downstream product serves as CAT's direct inducer, these two pathways are closely related. The exhaustion tracks and correlation of (≈ 0.90) show that the pro-oxidant plant metabolites target common upstream genomic signaling networks, particularly the Nrf2/ARE master regulatory circuit [12].

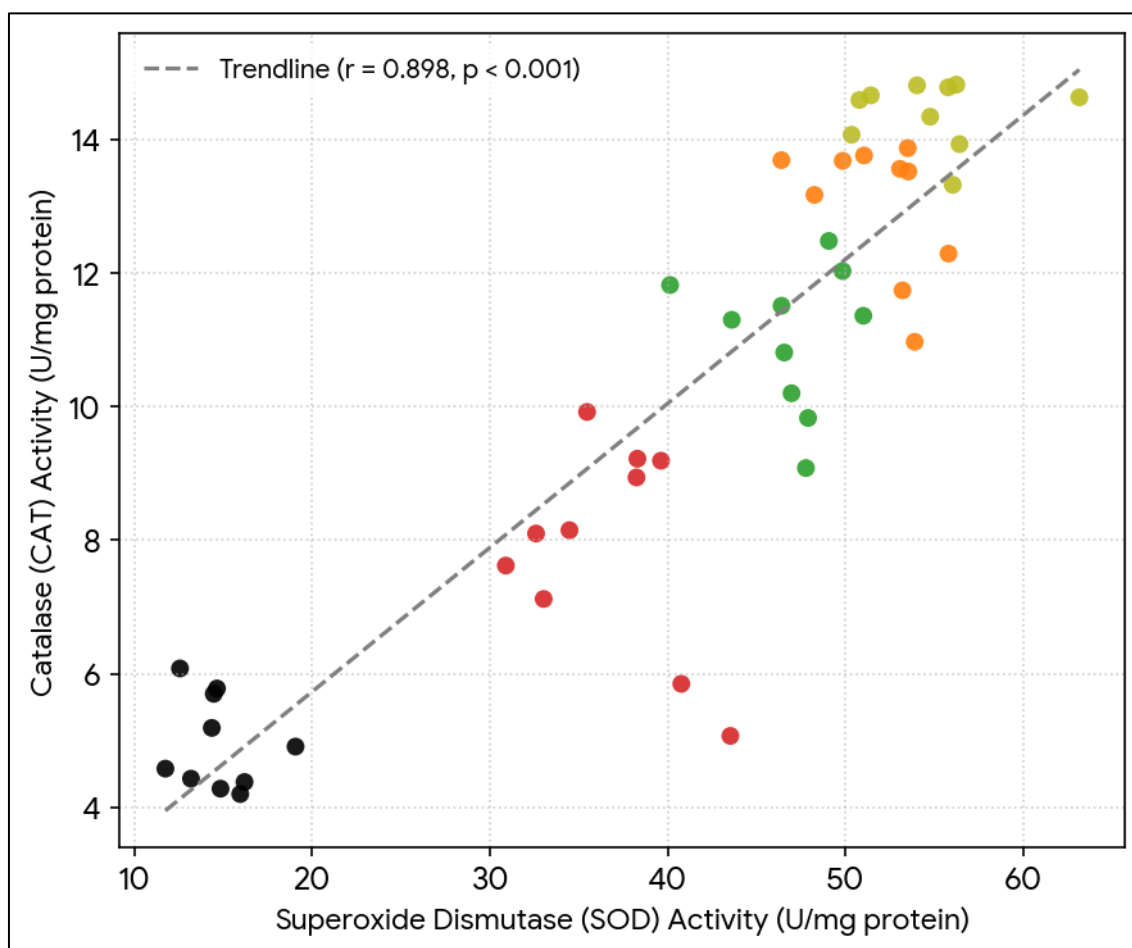


Figure 2: A scatter plot demonstrates that SOD and CAT activity are significantly positively correlated.

3.3 Macroscopic Manifestation of Cellular Injury: Liver Weight and OSI% vs. MDA

As seen in table 1, the matrix verified a very strong positive association at the tissue-organ level between the Organ Somatic Index (OSI%) and both MDA ($r = 0.919898$) and RCOSI ($r = 0.857191$). The shift from microscopic chemical damage to macroscopic pathogenic alterations is explained by this statistical connection. As seen in figure (3), MDA is the final byproduct of lipid peroxidation (LPO). Plant phenolics cause a self-propagating chain reaction of free radicals when they target the polyunsaturated fatty acids (PUFAs) of the mitochondrial membranes and hepatic endoplasmic reticulum [13]. This significant positive correlation is entirely consistent with the pathogenic frameworks proposed by Allameh study, which identified localized lipid peroxidation chain reactions as the primary causes of altered liver architecture and tissue hypertrophy. Malondialdehyde (MDA) is the final result of lipid peroxidation. When plant secondary compounds harm cell membranes and reduce selective permeability, an unregulated influx of extracellular fluid into the hepatocytes results in a pathological state known as cellular edema or ballooning degeneration [14].

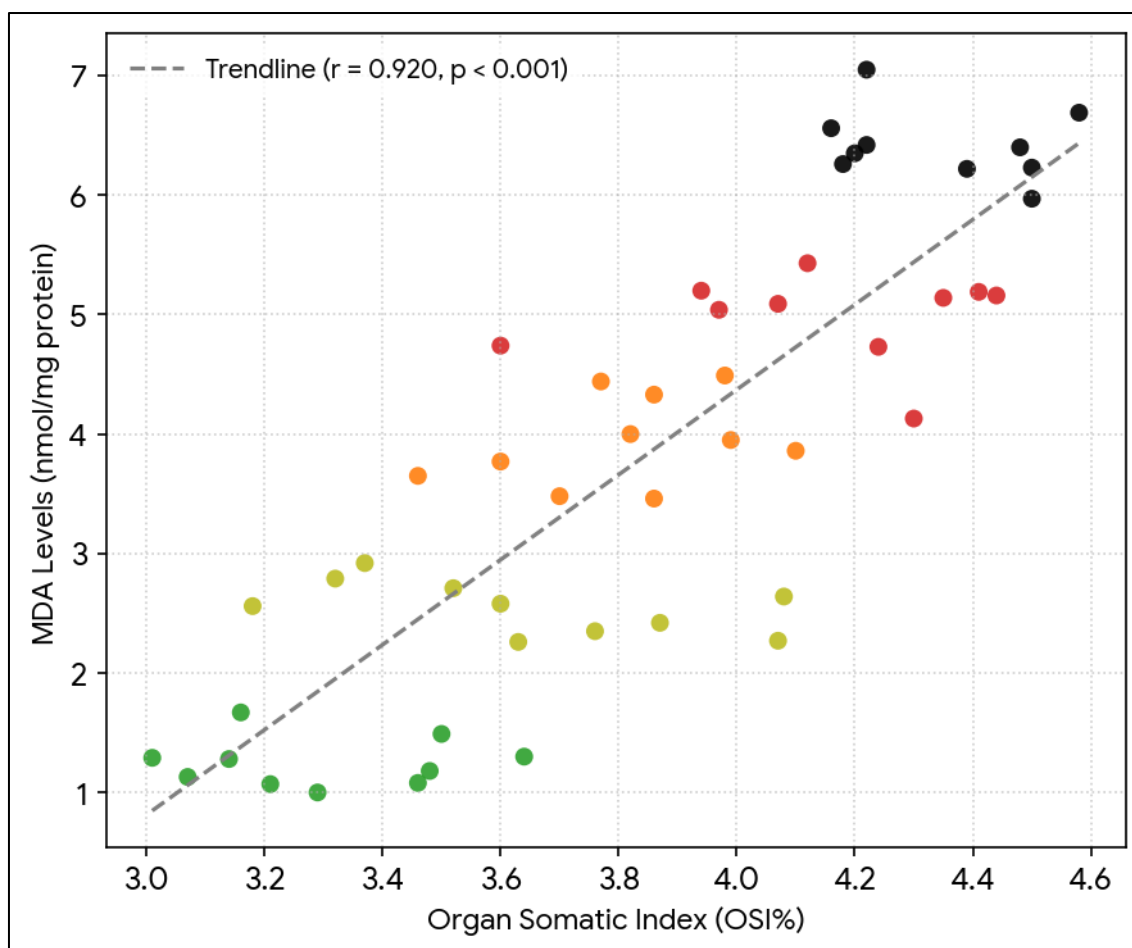


Figure 3: Scatter plot showing a strong positive correlation between OSI% and MDA levels.

3.4 Systemic Metabolic Disruption: MHC Coefficient Dynamics

Table 1 shows a strong positive association between the calculated Metabolic Homeostasis Coefficient (MHC) with MDA ($r = 0.88782$) and RCOSI ($r = 0.869226$). The animal's systemic bioenergetics and the localized hepatic oxidative stress are profoundly linked by this interaction. The disturbance of blood glucose in relation to total protein is efficiently monitored by the MHC index [15]. The animal's neuroendocrine axis is highly engaged during acute oxidative stress (high MDA), which causes a huge release of stress hormones, mainly catecholamines (adrenaline) and glucocorticoids (cortisol/corticosterone) as shown in figure (4). This substantial correlation supports the Contreras-Zentella study's conclusions that systemic glucose and lipid dysregulation is directly caused by localized organ-specific oxidative damage. When there is significant oxidative damage (high MDA), the animal's neuroendocrine system releases large amounts of stress hormones, primarily glucocorticoids and catecholamines. These hormones encourage rapid hepatic glycogenolysis and gluconeogenesis, overwhelming the circulatory system with glucose in an attempt to provide an immediate energy buffer against toxic shock. Lipid peroxidation simultaneously blocks insulin receptors on injured hepatocytes, leading to insulin resistance [16].

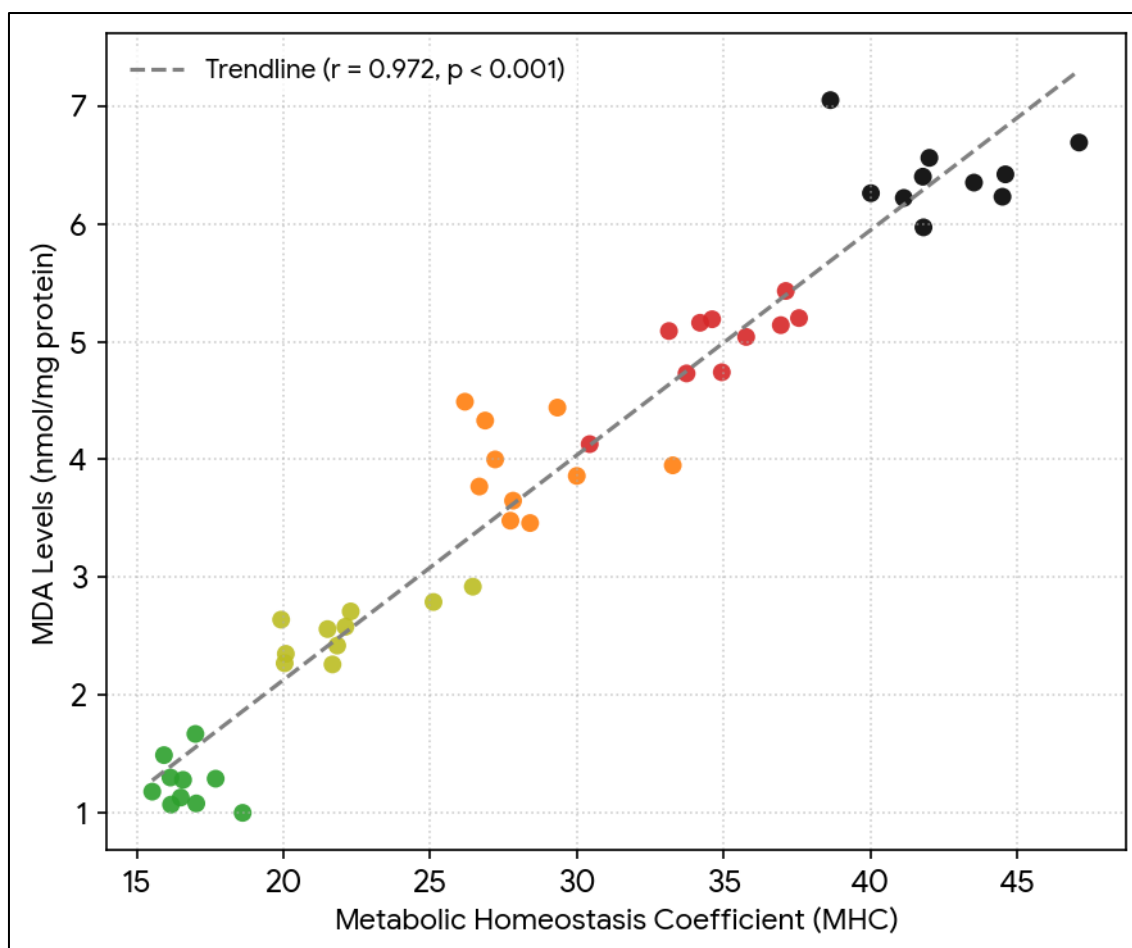


Figure 4: Scatter plot showing a severe positive correlation between MHC and MDA levels.

Table 1: Statistical Correlation Matrix Between Body/Organ Weights, Antioxidant Enzyme Activities, and Oxidative Stress Indices.

	Body_Weight_g	Liver_Weight_g	MDA_nmol_mg	SOD_U_mg	CAT_U_mg	RCOSI_Index	OSI_Percent	MHC_Coefficient
Body_Weight_g	1							
Liver_Weight_g	-0.07604	1						
MDA_nmol_mg	-0.05725	0.915078**	1					
SOD_U_mg	-0.00220	-0.77276***	-0.74993***	1				
CAT_U_mg	-0.01153	-0.71684***	-0.70071***	0.897968***	1			
RCOSI_Index	0.003187	0.857191**	0.817742**	-0.94163***	-0.82691***	1		
OSI_Percent	-0.45840***	0.837232**	0.919898**	-0.66844***	-0.62534***	0.726238***	1	

MHC_Coefficient	-0.06611	0.88782 ***	0.97192 ***	- 0.7588 2***	- 0.7977 4 ***	0.869226 ***	0.81822 6 ***	1
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Note/ *** Robust physiological couplings are indicated by a highly significant correlation at the 0.001 level (2-tailed, $p < 0.001$).

4 CONCLUSION

Mammalian homeostatic barriers are systematically dismantled by cumulative, high-dose exposure to plant defense phenolic chemicals, according to this study's empirical, multi-variable data. Mechanistically, the nearly perfect negative correlation ($r = -0.94163$, $p < 0.001$) between RCOSI and SOD shows that a large inflow of pro-oxidants causes the host's principal endogenous antioxidant lines to become "enzymatically exhausted." This defense failure allows self-propagating lipid peroxidation to degrade cell membranes, transforming microscopic cellular edema into macroscopic organ enlargement, as evidenced by the strong positive correlation between MDA and OSI_Percent ($r = 0.919898$). This liver architectural trauma causes a large systemic neuroendocrine stress response that leads to transient insulin resistance and hyperglycemia as indicated by the MHC_Coeff index. These findings ultimately demonstrate that the host's physiological defenses operate as a network of interrelated, co-regulated systems that abruptly collapse when specific chemical and xenobiotic exposure thresholds are exceeded.

STATEMENTS ON COMPLIANCE WITH ETHICAL STANDARDS

Disclosure of conflict of interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Statement of ethical approval

Ref.: CSEC/0926/0075, University of Baghdad, College of Science, Ethics Committee. csec@sc.uobaghdad.edu.iq

Data availability statement

The data used to support the findings of this study are available from the corresponding author upon reasonable request.

REFERENCES

- [1] Farghal M. Launching a new platform for Advances in Animal Sciences. *Adv Anim Sci.* 2026; 1(1): 2. doi: 10.65736/aas01011
- [2] Manswetha M, Gomathy G. Evaluation of Phytochemical Contents in the Methanolic Extract of Phyllanthus Niruri Leaves. *Int J Sci Res (IJSR).* 2024; 13(8): 584–590. doi: 10.21275/sr24806215748
- [3] Lahazi V, Taheri G, Jafarisani M. Antioxidant enzymes activity of *Ferula flabelliloba* and *Ferula diversivittata* extracts. *Turk J Biochem.* 2015; 40(4): 310–315. doi: 10.1515/tjb-2015-0016
- [4] Mahmoud MH, Wahba HMA, Mahmoud MH. Combating Increased Oxidative Stress and Hepatic Injury Induced by CCl₄ in Wistar Rats by A Polyphenol Rich Jam from Edible Plants. *Egypt J Chem.* 2025; 68(7): 339–349. doi: 10.21608/ejchem.2024.329059.10647
- [5] Alabi QK, Akomolafe RO. Kolaviron Diminishes Diclofenac-Induced Liver and Kidney Toxicity in Wistar Rats Via Suppressing Inflammatory Events, Upregulating Antioxidant Defenses, and Improving Hematological Indices. *Dose Response.* 2020; 18(1): 1559325819899256. doi: 10.1177/1559325819899256
- [6] Sajadimajd S, Khazaei M. Oxidative Stress and Cancer: The Role of Nrf2. *Curr Cancer Drug Targets.* 2018; 18(6): 538–557. doi: 10.2174/1568009617666171002144228
- [7] Abdel-Haleem SA, Ibrahim AY, Ismail RF, Shaffie NM, Hendawy SF, Omer EA. In-vivo hypoglycemic and hypolipidemic properties of *Tagetes lucida* alcoholic extract in streptozotocin-induced hyperglycemic Wistar albino rats. *Ann Agric Sci.* 2017; 62(2): 169–181. doi: 10.1016/j.aos.2017.11.005
- [8] Genslueckner S, Wernly B, Datz C, Aigner E. Iron, Oxidative Stress, and Metabolic Dysfunction—Associated Steatotic Liver Disease. *Antioxidants.* 2024; 13(2): 208. doi: 10.3390/antiox13020208
- [9] González P, Lozano P, Ros G, Solano F. Hyperglycemia and Oxidative Stress: An Integral, Updated and Critical Overview of Their Metabolic Interconnections. *Int J Mol Sci.* 2023; 24(11): 9352. doi: 10.3390/ijms24119352

- [10] Robertson RP. Perspective: Beta Cell Glucose Toxicity in Type 2 Diabetes: Nrf2 and the Endogenous Antioxidant Response. *Antioxidants*. 2026; 15(8): 997. doi: 10.3390/antiox15080997
- [11] Ma Q. Role of Nrf2 in Oxidative Stress and Toxicity. *Annu Rev Pharmacol Toxicol*. 2013; 53: 401–426. doi: 10.1146/annurev-pharmtox-011112-140320
- [12] Moon JS, Riopel M, Seo JB, Herrero-Aguayo V, Isaac R, Lee YS. HIF-2 α Preserves Mitochondrial Activity and Glucose Sensing in Compensating β -Cells in Obesity. *Diabetes*. 2022; 71(7): 1508–1524. doi: 10.2337/db21-0736
- [13] Adnan M, Badruddeen B, Arif M, Akhtar J, Islam A, Khan MI, Ahmad M, Noor G. Effect of Biochanin A and caffeic acid combination on hyperglycemia and oxidative stress in streptozotocin-induced diabetic rats. *J Phytonanotechnol Pharm Sci*. 2025; 5(3). doi: 10.54085/jpps.2025.5.3.3
- [14] Allameh A, Niayesh-Mehr R, Aliarab A, Sebastiani G, Pantopoulos K. Oxidative Stress in Liver Pathophysiology and Disease. *Antioxidants*. 2023; 12(9): 1653. doi: 10.3390/antiox12091653
- [15] Al-Sa'aidi JAA, Shoabith HJ. Sequential changes of serum and liver subcellular oxidants and antioxidant concentrations in silymarin treated male rats. *Iraqi J Vet Sci*. 2016; 30(1): 9–14. doi: 10.33899/ijvs.2016.116862
- [16] Contreras-Zentella ML, Hernández-Espinosa LC, Hernández-Muñoz R. Oxidative Stress in Liver Metabolic Dysfunction and Diseases, with a Focus on Hepatogenic Diabetes: Effect of Alcohol Consumption. *Antioxidants*. 2025; 14(12): 1494. doi: 10.3390/antiox14121494.