ISSN No:-2456-2165 https://doi.org/10.38124/ijisrt/26oct1153

Integrating Histological, Biochemical and Molecular Endpoints to Identify Predictors of Diet-Induced Hepatic Injury and the Protective Effects of Berry-Derived Bioactive Compounds

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Publication Date: 2026/10/23

Abstract: Diet-induced hepatic injury is a multifactorial pathological condition involving hepatic lipid accumulation, oxidative stress, inflammatory activation, mitochondrial dysfunction, and progressive hepatocellular damage. Conventional assessments frequently rely on isolated biochemical biomarkers or histopathological observations, potentially overlooking complex interactions among tissue morphology, metabolic disturbances, and molecular signalling pathways. This study proposes an integrated experimental and computational framework for identifying predictors of diet-induced hepatic injury and evaluating the hepatoprotective effects of berry-derived bioactive compounds. A novel Berry-Hepatic Multimodal Predictive Algorithm (BHMPA) is introduced to integrate quantitative histological, biochemical, and molecular endpoints through adaptive feature weighting, attention-based multimodal fusion, pathway-informed regularization, and explainable machine learning. The proposed experimental design incorporates normal-diet controls, high-fat/high-fructose diet-induced hepatic injury groups, and dietary intervention groups receiving standardized anthocyanin-rich blueberry, blackberry, or bilberry extracts at predetermined concentrations. Histological endpoints include hepatic steatosis, inflammatory infiltration, hepatocellular ballooning, and fibrosis severity, while biochemical assessments incorporate alanine aminotransferase, aspartate aminotransferase, hepatic triglycerides, malondialdehyde, superoxide dismutase, glutathione peroxidase, and inflammatory cytokines. Molecular investigations target Nrf2/HO-1, NF-κB, SREBP-1c, PPAR-α, and apoptosis-associated signalling pathways. The BHMPA framework combines supervised hepatic injury classification, continuous histopathological severity prediction, and estimation of intervention-associated protection scores within a multitask optimization architecture. Its predictive performance will be systematically compared with Random Forest, Support Vector Machine, Extreme Gradient Boosting (XGBoost), Elastic Net, and Data Integration Analysis for Biomarker Discovery Using Latent Components (DIABLO). Model evaluation will employ nested cross-validation, animal-level data partitioning, external validation where feasible, area under the receiver operating characteristic curve, F1-score, balanced accuracy, root mean square error, calibration analysis, and statistical significance testing. Comparative bar charts, receiver operating characteristic curves, biomarker correlation heatmaps, dose-response graphs, and SHAP-based feature attribution plots will quantify predictive accuracy, biological associations, and treatment-related responses. The study hypothesizes that biologically constrained multimodal integration will improve predictive discrimination, biomarker stability, and interpretability relative to conventional single-modality and established machine learning approaches. The proposed framework offers a reproducible strategy for investigating nutritional hepatoprotection, identifying mechanistically relevant hepatic injury biomarkers, and prioritizing berry-derived bioactive compounds for further experimental validation.

Keywords: *Diet-Induced Hepatic Injury; Histological Endpoints; Biochemical Biomarkers; Molecular Endpoints; Berry-Derived Bioactive Compounds.*

How to Cite: Azeezat Folakemi Abdus-salam (2026) Integrating Histological, Biochemical and Molecular Endpoints to Identify Predictors of Diet-Induced Hepatic Injury and the Protective Effects of Berry-Derived Bioactive Compounds. *International Journal of Innovative Science and Research Technology*, 11(10), 1456-1476. https://doi.org/10.38124/ijisrt/26oct1153

I. INTRODUCTION

➢ *Background to Diet-Induced Hepatic Injury and Metabolic Dysfunction*

Diet-induced hepatic injury constitutes a significant metabolic pathology arising from prolonged exposure to energy-dense diets containing excessive saturated fats, refined carbohydrates, and fructose. These dietary conditions disrupt hepatic lipid homeostasis, insulin signalling, mitochondrial oxidative metabolism, and inflammatory regulation, promoting intracellular triglyceride accumulation and progressive hepatocellular dysfunction. Younossi et al.

ISSN No:-2456-2165 https://doi.org/10.38124/ijisrt/26oct1153

(2016) established the substantial global burden of nonalcoholic fatty liver disease (NAFLD), reporting a pooled prevalence of 25.24% in the populations examined, demonstrating its importance as a metabolic health concern. The contemporary clinical terminology, metabolic dysfunction-associated steatotic liver disease (MASLD), reflects the central contribution of cardiometabolic abnormalities to hepatic lipid accumulation. Mechanistically, excessive hepatic fatty acid influx, increased de novo lipogenesis, inadequate β-oxidation, and impaired lipid export collectively disturb hepatocyte metabolic equilibrium. These alterations provide the physiological foundation for evaluating dietary hepatic injury through integrated biochemical measurements, histological examination, and molecular investigations.

Friedman et al. (2018) demonstrated that hepatic disease progression involves interactions among lipotoxicity, oxidative stress, inflammatory signalling, and fibrogenic activation. Consequently, assessments restricted to serum aminotransferases may incompletely represent underlying tissue damage and molecular disturbances. The present study addresses this limitation by integrating hepatic steatosis scores, alanine aminotransferase activity, oxidative stress biomarkers, inflammatory mediators, and lipid-regulatory gene expression within the proposed Berry-Hepatic Multimodal Predictive Algorithm (BHMPA). Controlled dietary intervention groups receiving anthocyanin-rich berry extracts will facilitate comparative evaluation of injury-associated biomarkers and potential hepatoprotective responses. This integrated design establishes the biological basis for identifying predictors of hepatic injury severity while distinguishing biochemical improvement from demonstrable histopathological recovery.

- *Pathophysiological Mechanisms of Hepatic Steatosis, Oxidative Stress, Inflammation, and Hepatocellular Damage*

Diet-induced hepatic injury develops through interconnected metabolic disturbances involving excessive lipid deposition, mitochondrial dysfunction, oxidative stress, and inflammatory activation. Buzzetti et al. (2016) described hepatic disease progression using a multiple-hit framework in which insulin resistance, adipose-derived mediators, nutritional factors, intestinal microbiota, and genetic susceptibility interact to determine pathological severity. Excessive hepatic fatty acid availability stimulates triglyceride accumulation and increases the production of potentially toxic lipid intermediates, including diacylglycerols and ceramides. Concurrent mitochondrial substrate overload promotes reactive oxygen species generation, lipid peroxidation, and impaired respiratory efficiency. Elevated malondialdehyde concentrations, reduced superoxide dismutase activity, and diminished glutathione-dependent antioxidant protection therefore represent relevant biochemical indicators. These disturbances can compromise hepatocyte membrane integrity and increase circulating alanine and aspartate aminotransferase activities, although enzyme concentrations do not consistently correspond to histological disease severity.

Inflammatory progression involves activation of Kupffer cells, recruitment of circulating immune cells, and stimulation of nuclear factor-kappa B signalling, resulting in increased expression of tumour necrosis factor-alpha, interleukin-6, and other inflammatory mediators. Tilg and Moschen (2010) emphasized that inflammatory activity may arise through several parallel mechanisms rather than a uniform sequence following steatosis. Persistent injury promotes hepatocellular ballooning, apoptotic signalling, stellate-cell activation, and extracellular matrix deposition. Accordingly, the proposed investigation will quantify hepatic lipid accumulation, oxidative stress, inflammatory mediators, and molecular pathway activity alongside histological lesions. Integrating these interdependent endpoints through BHMPA is intended to identify predictive biomarker combinations and characterize whether berry-derived interventions modify several pathological mechanisms simultaneously.

- *Histological, Biochemical, and Molecular Indicators of Diet-Induced Liver Injury*

Reliable characterization of diet-induced hepatic injury requires complementary measurements that represent structural tissue alterations, hepatocellular biochemical disturbances, and regulatory molecular processes. Histopathological assessment permits direct examination of macrovesicular steatosis, lobular inflammation, hepatocellular ballooning, and fibrosis. Kleiner et al. (2005) developed a validated histological scoring system incorporating steatosis, lobular inflammation, and ballooning into the NAFLD Activity Score, providing an established framework for semiquantitative evaluation. Bedossa et al. (2012) subsequently developed a histopathological classification algorithm based on steatosis, ballooning, and lobular inflammation, reinforcing the importance of combining morphological indicators when characterizing steatohepatitis. For the present investigation, hematoxylin-and-eosin-stained sections will support assessment of tissue architecture, while appropriate lipid and collagen staining will quantify hepatic fat deposition and fibrosis. Digital image analysis will additionally enable estimation of lipid-droplet area, inflammatory-cell density, and collagen-positive tissue fractions.

Biochemical evaluation will incorporate circulating alanine aminotransferase, aspartate aminotransferase, hepatic triglycerides, malondialdehyde, superoxide dismutase, and glutathione peroxidase. Molecular measurements will examine Nrf2/HO-1 antioxidant regulation, NF-κB-mediated inflammation, SREBP-1c-associated lipogenesis, PPAR-α-mediated fatty acid oxidation, and apoptosis-related signalling. Rather than treating these measurements as independent disease indicators, BHMPA will encode histological, biochemical, and molecular variables as complementary feature matrices. The analytical framework will identify statistically stable predictors of hepatic injury severity and assess whether biomarker combinations improve discrimination between normal-diet, diet-injured, and berry-treated experimental groups. Histological scoring will remain an independently assessed reference outcome to prevent circular validation.

ISSN No:-2456-2165 https://doi.org/10.38124/ijisrt/26oct1153

- *Berry-Derived Bioactive Compounds, Anthocyanins, and Their Potential Hepatoprotective Mechanisms*

Berry-derived bioactive compounds, particularly anthocyanins, phenolic acids, and flavonols, constitute potentially valuable dietary agents for modulating hepatic metabolic disturbances associated with excessive fat consumption. Anthocyanins such as cyanidin-3-glucoside, delphinidin glycosides, and malvidin derivatives exhibit biological activities associated with antioxidant defence, inflammatory regulation, and cellular energy metabolism. Tang et al. (2015) demonstrated that purified anthocyanins derived from bilberry and black currant attenuated steatohepatitis and hepatic mitochondrial dysfunction in mice subjected to a methionine- and choline-deficient diet. Their findings implicated AMP-activated protein kinase and PGC-1α-associated mitochondrial regulation in the observed protective response. These observations support investigating whether anthocyanin exposure reduces hepatic lipid accumulation, improves mitochondrial homeostasis, and modulates pathways responsible for hepatocellular injury. Nevertheless, differences between dietary models require careful consideration when extrapolating biological effects.

Pemmari et al. (2022) reported that dried bilberry supplementation attenuated high-fat-diet-associated increases in alanine aminotransferase and several inflammatory mediators, despite not significantly altering weight gain. This distinction suggests that improvements in hepatic physiology may occur independently of substantial changes in overall body mass. The proposed investigation will therefore compare standardized blueberry, blackberry, and bilberry extracts using anthocyanin concentration, administered dose, biochemical response, histological improvement, and molecular pathway activity. Particular emphasis will be placed on Nrf2/HO-1, NF-κB, SREBP-1c, and PPAR-α signalling. BHMPA will integrate these measurements to estimate intervention-associated protection and identify combinations of biomarkers that most consistently characterize treatment responsiveness, without assuming equivalent efficacy across different berry species or extract preparations.

- *Applications of Machine Learning, Multimodal Data Integration, and Predictive Biomarker Discovery in Hepatic Injury Assessment*

Machine learning provides computational methods for examining nonlinear relationships, correlated predictors, and complex interactions among heterogeneous biological measurements. Conventional statistical approaches may inadequately capture associations linking hepatic histopathology, circulating biochemical indicators, and molecular signalling networks when the relationships are highly nonlinear or involve interacting variables. Rohart et al. (2017) introduced the mixOmics framework for multivariate feature selection and integration across different molecular datasets, demonstrating the methodological importance of identifying shared biological patterns rather than analysing individual datasets independently. In diet-induced hepatic injury studies, relevant inputs may include quantitative hepatic steatosis, inflammatory-cell infiltration, aminotransferase activities, oxidative stress indicators, and gene-expression measurements. Integrating these heterogeneous features could improve the identification of predictive biomarker signatures, provided sample size, data quality, and validation procedures adequately support the resulting model complexity.

Singh et al. (2019) developed DIABLO, a supervised multiblock integration approach that identifies discriminative molecular signatures across multiple omics datasets. This methodology provides a relevant comparator for evaluating the proposed BHMPA architecture, although the present application combines histological and conventional biochemical endpoints with molecular measurements rather than relying exclusively on omics data. BHMPA is designed to incorporate modality-specific feature processing, adaptive attention weights, biological pathway constraints, and multitask predictions of hepatic injury classification, histological severity, and intervention-associated protection. Comparative evaluation against Random Forest, Support Vector Machine, XGBoost, Elastic Net, and DIABLO will use animal-level nested cross-validation, calibration analysis, classification metrics, and regression errors. Feature-attribution analyses will additionally assess biological plausibility, predictor stability, and the incremental information contributed by each measurement modality.

- *Limitations of Conventional Hepatic Injury Assessment, Single-Modality Prediction, and Existing Computational Algorithms*

Conventional hepatic injury assessment remains constrained by the incomplete correspondence between circulating biomarkers, tissue-level pathology, and intracellular molecular disturbances. Serum aminotransferases provide useful evidence of hepatocellular injury but cannot independently establish the distribution of lipid droplets, inflammatory infiltration, hepatocyte ballooning, or fibrosis. Histopathological assessment provides important structural information but introduces sampling limitations and variability arising from observer-dependent interpretation. Takahashi et al. (2023) identified substantial interest in artificial intelligence-assisted histopathological assessment as a means of improving quantitative consistency in fatty liver disease evaluation. Nevertheless, image-based algorithms may remain biologically incomplete when biochemical and molecular measurements are excluded. Similarly, isolated molecular biomarkers may reflect pathway activation without establishing whether corresponding structural damage has occurred. These limitations justify integrating multiple biological domains rather than relying on a single diagnostic or predictive modality.

Computational prediction introduces additional challenges involving small experimental datasets, correlated predictors, class imbalance, inappropriate validation procedures, and model overfitting. Andaur Navarro et al. (2021) reported that 87% of the machine learning prediction-model analyses examined in their systematic review had a high risk of bias, highlighting important methodological weaknesses in model development. For the proposed hepatic injury investigation, these concerns are especially relevant

ISSN No:-2456-2165 https://doi.org/10.38124/ijisrt/26oct1153

because high-dimensional molecular features may substantially exceed the number of experimental animals. BHMPA will therefore incorporate constrained feature selection, pathway-informed regularization, nested cross-validation, and animal-level data separation. Its comparative assessment will include calibration, confidence intervals, ablation analysis, and independent validation where feasible. Predictive superiority will be established only if the model demonstrates reproducible improvements against appropriately optimized benchmark algorithms.

➢ *Research Problem, Knowledge Gaps, and Justification for the Proposed Berry-Hepatic Multimodal Predictive Algorithm (BHMPA)*

Despite expanding research on dietary hepatotoxicity and nutritional hepatoprotection, identifying biologically meaningful predictors that simultaneously represent hepatic tissue injury, metabolic dysfunction, and molecular regulation remains methodologically challenging. Existing experimental studies frequently examine the effects of bioactive compounds through individual endpoints or limited biomarker panels, making it difficult to establish which combinations best predict histological injury severity and intervention-associated improvement. Xu et al. (2025) demonstrated that blueberry anthocyanins improved hepatic lipid accumulation and oxidative stress indicators in high-fat-diet-fed mice, while integrated molecular investigations identified associations involving intestinal microbiota and bile acid metabolism. Such findings establish the relevance of interconnected metabolic pathways but do not independently demonstrate the predictive superiority of a histological, biochemical, and molecular fusion algorithm. A systematic computational framework is therefore required to evaluate cross-domain biomarker relationships and identify reproducible indicators of hepatic injury and treatment response.

The proposed BHMPA addresses this research problem through modality-specific feature encoding, adaptive multimodal fusion, pathway-informed regularization, and simultaneous prediction of injury classification, histological severity, and treatment-associated protection. Its evaluation will extend beyond discrimination accuracy to include model calibration, biological interpretability, and statistical comparisons with established algorithms. Vickers and Elkin (2006) demonstrated the importance of decision-curve analysis for evaluating the practical usefulness of predictive models beyond conventional accuracy measures. Accordingly, the present study will assess whether BHMPA provides meaningful predictive improvement through receiver operating characteristic analysis, balanced accuracy, root mean square error, calibration plots, and decision-curve analysis where a defensible decision threshold exists. Comparative results will establish whether the proposed integration strategy offers measurable advantages in identifying hepatic injury predictors and characterizing the hepatoprotective potential of berry-derived bioactive compounds.

➢ *Research Objectives, Research Questions, Hypotheses, and Scientific Contributions*

- *Research Objectives*

The principal objective of this study is to develop and evaluate an integrated experimental and computational framework capable of identifying reliable predictors of diet-induced hepatic injury and quantifying the potential hepatoprotective effects of berry-derived bioactive compounds through the proposed Berry-Hepatic Multimodal Predictive Algorithm (BHMPA).

- *The Specific Objectives are to:*

✓ Quantify histological, biochemical, and molecular alterations associated with diet-induced hepatic injury using hepatic steatosis, inflammatory infiltration, hepatocellular ballooning, fibrosis, serum aminotransferases, oxidative stress biomarkers, and molecular signalling indicators.
✓ Investigate the comparative hepatoprotective effects of standardized blueberry, blackberry, and bilberry bioactive extracts on hepatic lipid accumulation, oxidative stress, inflammatory regulation, mitochondrial function, and injury-associated molecular pathways.
✓ Develop the novel BHMPA algorithm using modality-specific feature encoding, adaptive attention-based fusion, biologically informed pathway regularization, and multitask predictive optimization.
✓ Identify and rank the most influential histological, biochemical, and molecular predictors of hepatic injury severity and berry-associated treatment responsiveness using explainable machine learning, feature-attribution analysis, and biomarker interaction modelling.
✓ Compare BHMPA against Random Forest, Support Vector Machine, XGBoost, Elastic Net, and DIABLO using AUROC, F1-score, balanced accuracy, RMSE, calibration, computational performance, and statistically validated predictive differences.
✓ Evaluate the generalizability, robustness, and biological interpretability of BHMPA through nested cross-validation, multimodal feature-ablation experiments, sensitivity analysis, dose-response modelling, and independent validation where feasible.

- *Research Questions*

✓ What histological, biochemical, and molecular alterations most reliably characterize the development and severity of diet-induced hepatic injury?
✓ How do blueberry, blackberry, and bilberry bioactive compounds differ in their effects on hepatic steatosis, oxidative stress, inflammatory responses, and molecular pathways associated with hepatocellular injury?
✓ Can the proposed BHMPA algorithm effectively integrate heterogeneous histological, biochemical, and molecular datasets to predict hepatic injury classification, disease severity, and treatment-associated hepatoprotection?
✓ Which biomarkers and molecular pathway interactions provide the greatest predictive contributions to hepatic

ISSN No:-2456-2165 https://doi.org/10.38124/ijisrt/26oct1153

injury assessment and berry-derived intervention responsiveness?
- ✓ Does BHMPA achieve statistically significant improvements in predictive accuracy, error reduction, calibration, and interpretability compared with Random Forest, Support Vector Machine, XGBoost, Elastic Net, and DIABLO?
- ✓ To what extent do multimodal feature integration, adaptive weighting, pathway-informed regularization, and multitask optimization improve the robustness and generalizability of the proposed algorithm across experimental conditions?

- *Research Hypotheses*

The following hypotheses will be examined using appropriate statistical and computational validation procedures.

- ✓ H_{01}: There are no statistically significant differences in the measured histological, biochemical, and molecular endpoints between normal-diet and hepatic injury-induced experimental groups.
- ✓ H_{11}: Diet-induced hepatic injury produces statistically significant alterations in histological, biochemical, and molecular endpoints relative to normal-diet controls.
- ✓ H_{02}: Berry-derived bioactive interventions do not significantly improve hepatic histopathology, biochemical injury markers, oxidative stress indicators, or molecular pathway activity compared with untreated diet-injured controls.
- ✓ H_{12}: At least one standardized berry-derived bioactive intervention significantly improves one or more prespecified hepatic injury endpoints compared with untreated diet-injured controls.
- ✓ H_{03}: The proposed BHMPA algorithm does not achieve significantly better predictive performance than the strongest appropriately optimized benchmark algorithm.
- ✓ H_{13}: The proposed BHMPA algorithm achieves significantly better predictive performance than the strongest appropriately optimized benchmark algorithm on prespecified evaluation metrics.
- ✓ H_{04}: Integrating histological, biochemical, and molecular endpoints does not significantly improve hepatic injury prediction compared with the best-performing single-modality model.
- ✓ H_{14}: Integrated histological, biochemical, and molecular endpoints significantly improve hepatic injury prediction compared with the best-performing single-modality model.
- ✓ H_{05}: Incorporating pathway-informed regularization and adaptive multimodal weighting does not significantly improve BHMPA predictive performance or biomarker-selection stability.
- ✓ H_{15}: Incorporating pathway-informed regularization and adaptive multimodal weighting significantly improves BHMPA predictive performance or biomarker-selection stability.

- *Scientific Contributions*

The anticipated scientific contributions of this research comprise the following:

- ✓ Development of a novel multimodal predictive architecture: The research will introduce BHMPA as a computational framework designed to integrate tissue morphology, biochemical measurements, and molecular pathway activity for simultaneous hepatic injury classification, severity estimation, and prediction of intervention-associated responses.
- ✓ Identification of integrated hepatic injury biomarker signatures: The study will establish a systematic analytical procedure for ranking candidate predictors across hepatic steatosis, inflammatory infiltration, oxidative stress, lipid metabolism, antioxidant signalling, and apoptosis-associated molecular indicators.
- ✓ Quantitative assessment of berry-derived hepatoprotection: Standardized berry interventions will be evaluated through comparative biochemical measurements, histological scoring, molecular pathway analysis, and dose-response relationships, allowing protective effects to be examined across multiple biological endpoints.
- ✓ Development of a biologically informed feature-integration strategy: BHMPA will incorporate molecular pathway relationships into its regularization structure while applying adaptive feature weighting to investigate whether biological constraints improve predictive consistency and interpretability.
- ✓ Comparative assessment against established computational algorithms: The research will provide a reproducible experimental benchmarking procedure comparing BHMPA with Random Forest, Support Vector Machine, XGBoost, Elastic Net, and DIABLO, including discrimination, calibration, prediction error, statistical uncertainty, and feature-ablation analyses.
- ✓ An explainable framework for hepatic injury prediction: The investigation will incorporate feature-attribution techniques, biomarker interaction analysis, and model sensitivity testing to distinguish influential predictive variables from potentially redundant or unstable biomarker associations.
- ✓ A reproducible foundation for nutritional hepatoprotection research: The proposed computational and experimental workflow will support independent evaluation of predictive models, cross-study comparison of hepatic injury endpoints, and future investigation of bioactive dietary compounds in metabolic liver disease.

II. LITERATURE REVIEW

➢ *Experimental Models of Diet-Induced Hepatic Injury, Histopathological Alterations, and Quantitative Liver Tissue Assessment*

Experimental investigation of diet-induced hepatic injury requires reproducible animal models capable of representing metabolic disturbances, inflammatory progression, and hepatocellular structural alterations. Im et al. (2021), through a systematic evaluation of 3,920 rodent models, established that high-fat, high-fructose dietary protocols most closely reproduce several metabolic and histological characteristics of human nonalcoholic fatty liver disease as represented in figure 1. Brunt (2010) identified macrovesicular steatosis, lobular inflammation,

ISSN No:-2456-2165 https://doi.org/10.38124/ijisrt/26oct1153

hepatocellular ballooning, and fibrosis as important morphological indicators of progressive hepatic pathology. Consequently, the proposed investigation will employ controlled dietary groups incorporating normal-diet controls, untreated high-fat/high-fructose-fed animals, and berry-extract intervention groups. Histological assessment will involve hematoxylin-and-eosin staining, appropriate collagen staining, and quantitative digital image analysis. Steatosis area fraction, inflammatory-cell density, hepatocyte ballooning frequency, and fibrosis-associated collagen deposition will constitute measurable endpoints. Standardized tissue sampling, blinded histopathological scoring, and controlled image-acquisition procedures will minimize technical variability and observer-dependent measurement errors.

Quantitative histopathology additionally requires systematic data acquisition and reliable measurement traceability. Frank (2026) discussed healthcare quality measurement frameworks that provide a general methodological rationale for consistent data collection and performance assessment. Similarly, the biosensor-monitoring work of Atalor et al. (2023) illustrates the broader importance of integrating measurable biological signals, although its clinical application differs from experimental liver pathology. Idika et al. (2023) examined digital twin modelling in cyber-physical systems, providing a cross-domain computational analogy for representing interconnected system states. Building on these methodological principles, BHMPA will combine quantitative hepatic tissue measurements with biochemical and molecular indicators, using standardized feature extraction and animal-level data partitioning. Histological injury scores will serve as independently assessed reference outcomes, enabling comparative evaluation of predicted disease severity without introducing outcome leakage into model training.

Figure 1 presents an integrated experimental framework for evaluating diet-induced hepatic injury and the potential hepatoprotective effects of berry-derived bioactive compounds through three interconnected components. The first component describes experimental injury induction using high-fat/high-fructose diets in C57BL/6J mice, incorporating normal-diet controls, untreated diet-injured animals, and groups receiving standardized blueberry, blackberry, and bilberry extracts at predetermined doses. The second component characterizes hepatic histopathological alterations, including macrovesicular steatosis, lobular inflammatory infiltration, hepatocellular ballooning, and fibrosis, which collectively reflect the structural manifestations of progressive liver injury. The third component establishes quantitative tissue assessment procedures involving hematoxylin-and-eosin staining, collagen-specific staining, digital microscopy, histopathological scoring, and computational extraction of morphological features such as lipid-droplet area, inflammatory-cell density, and collagen deposition. These independently measured histological indicators are subsequently integrated with biochemical and molecular biomarkers through the proposed Berry-Hepatic Multimodal Predictive Algorithm (BHMPA). The framework incorporates adaptive feature weighting, multimodal data fusion, and predictive modelling to identify influential hepatic injury indicators, estimate disease severity, and evaluate intervention-associated histological improvement. By connecting controlled dietary exposure, tissue-level pathological characterization, and quantitative computational analysis, the framework establishes a systematic foundation for evaluating hepatic injury mechanisms and investigating berry-associated hepatoprotection while maintaining independent validation of predictive outcomes.

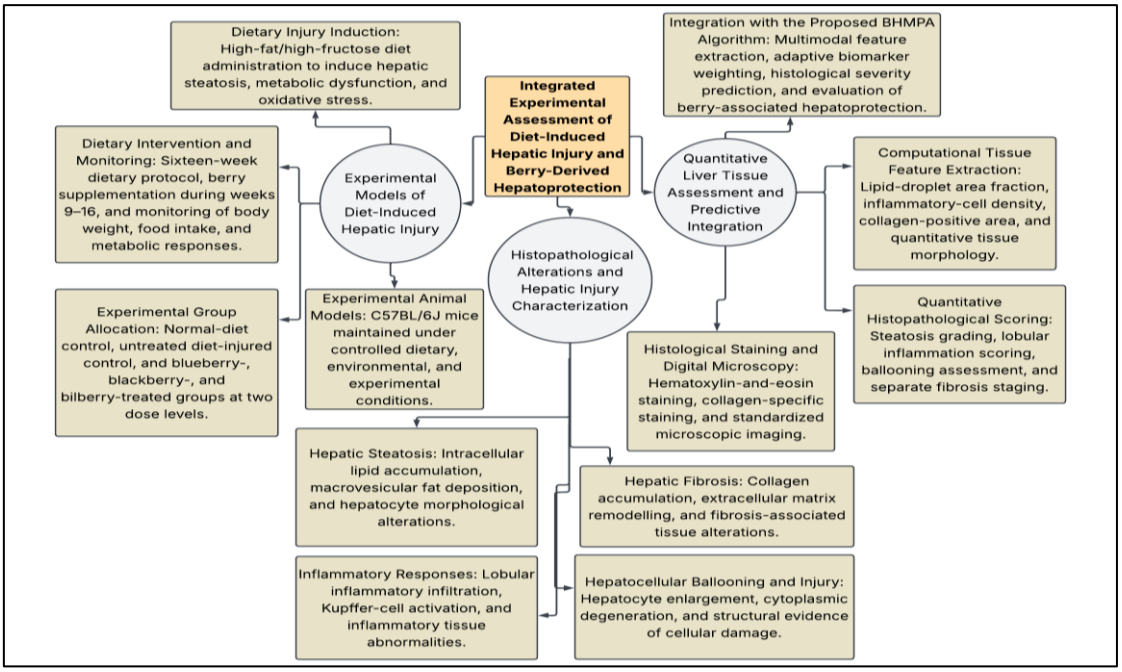


Fig 1 Integrated Framework Illustrating Experimental Models, Histopathological Alterations, and Quantitative Liver Tissue Assessment in Diet-Induced Hepatic Injury.

ISSN No:-2456-2165 https://doi.org/10.38124/ijisrt/26oct1153

- *Biochemical Indicators of Hepatocellular Injury, Lipid Peroxidation, Antioxidant Defence, and Metabolic Dysregulation*

Biochemical characterization of diet-induced hepatic injury requires simultaneous investigation of hepatocellular membrane integrity, oxidative stress, antioxidant defence, and lipid metabolic regulation. Hodson and Gunn (2019) established that hepatic triglyceride accumulation reflects disturbances in fatty acid uptake, de novo lipogenesis, oxidation, and lipid export as represented in figure 2. Yki-Järvinen et al. (2021) further demonstrated the importance of dietary saturated fats and excess carbohydrate consumption in regulating intrahepatic triglyceride accumulation and metabolic dysfunction. Consequently, serum alanine aminotransferase and aspartate aminotransferase will be evaluated alongside hepatic triglycerides, cholesterol, malondialdehyde, superoxide dismutase, glutathione peroxidase, and reduced glutathione. Elevated malondialdehyde indicates enhanced lipid peroxidation, whereas reduced antioxidant enzyme activity may reflect impaired cellular protection against reactive oxygen species. These biochemical measurements will support evaluation of berry-associated hepatoprotection while recognizing that individual markers are influenced by biological variability and may not independently establish histological recovery.

Dietary characteristics and analytical measurement quality also influence biochemical interpretation. Idoko et al. (2024) examined associations between dietary patterns and metabolic health outcomes, illustrating the broader importance of nutritional exposure assessment. Darko et al. (2024) addressed dietary quality and nutritional outcomes within sustainable food systems, providing contextual rather than liver-specific evidence. Frank (2026) discussed predictive analytics in healthcare inventory management, highlighting general considerations of data completeness and measurement availability. Accordingly, the proposed experimental framework will control dietary composition, exposure duration, sampling conditions, and biochemical assay procedures. BHMPA will normalize biochemical variables and evaluate their relationships with hepatic steatosis, inflammatory activity, and molecular pathway expression. Correlation heatmaps, treatment-group comparisons, and biomarker-ablation analyses will determine whether combined biochemical measurements provide incremental predictive information beyond individual aminotransferases or oxidative stress indicators.

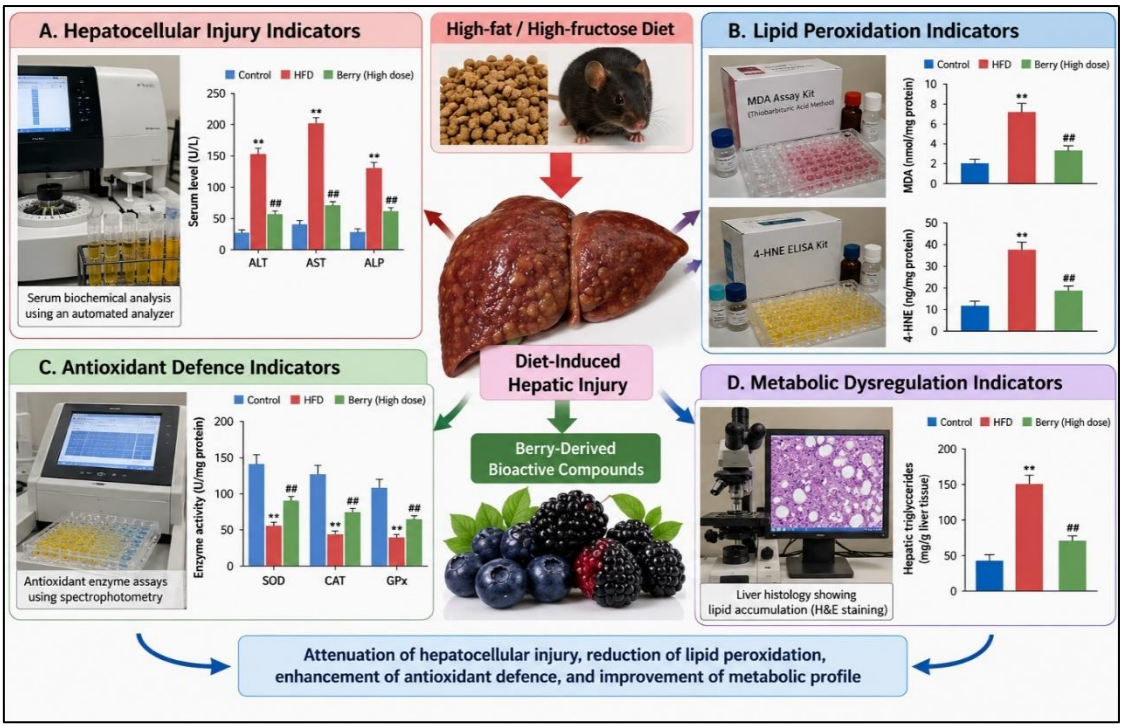


Fig 2 Integrated Assessment of Biochemical Indicators of Hepatocellular Injury, Lipid Peroxidation, Antioxidant Defence, and Metabolic Dysregulation Following Berry-Derived Bioactive Compound Intervention.

Figure 2 illustrates the interconnected biochemical mechanisms underlying diet-induced hepatic injury and the potential protective effects of berry-derived bioactive compounds. The illustration presents four principal biochemical domains associated with high-fat/high-fructose dietary exposure, including hepatocellular injury, lipid peroxidation, antioxidant defence, and metabolic dysregulation. Hepatocellular injury is characterized by increased serum alanine aminotransferase (ALT), aspartate aminotransferase (AST), and alkaline phosphatase (ALP), reflecting disturbances in hepatocyte integrity and liver function. Lipid peroxidation is represented by elevated malondialdehyde (MDA) and 4-hydroxynonenal (4-HNE), indicating oxidative degradation of membrane lipids and increased reactive oxygen species activity. Antioxidant defence is characterized by reduced superoxide dismutase (SOD), catalase (CAT), and glutathione peroxidase (GPx) activities, suggesting compromised cellular protection

ISSN No:-2456-2165 https://doi.org/10.38124/ijisrt/26oct1153

against oxidative damage. Metabolic dysregulation is illustrated through increased hepatic triglyceride accumulation, consistent with disturbances in fatty acid synthesis, oxidation, and lipid export. The berry-derived bioactive compounds, particularly anthocyanins and associated polyphenolic metabolites, are depicted as potential modulators of these abnormalities through reduced hepatocellular enzyme release, suppression of lipid peroxidation, restoration of antioxidant enzyme activities, and improvement of hepatic lipid metabolism. These interconnected biochemical endpoints provide the experimental foundation for the proposed Berry-Hepatic Multimodal Predictive Algorithm (BHMPA), which integrates biochemical, histological, and molecular measurements to identify dominant predictors of hepatic injury severity and assess intervention-associated hepatoprotection. The image represents a conceptual illustration of expected biological responses rather than independently verified experimental findings.

- *Molecular Mechanisms of Hepatic Injury and Berry-Mediated Hepatoprotection Through Nrf2/HO-1, NF-κB, SREBP-1c, PPAR-α, and Apoptotic Signalling Pathways*

Diet-induced hepatic injury involves interconnected molecular pathways regulating antioxidant defence, inflammatory signalling, lipid accumulation, and programmed hepatocyte death. Cuadrado et al. (2019) described the central function of Nrf2 and its regulatory partner KEAP1 in maintaining redox and metabolic homeostasis. Nrf2 activation promotes transcription of cytoprotective genes, including heme oxygenase-1, while dysregulated NF-κB signalling contributes to inflammatory mediator expression as presented in table 1. Wree et al. (2013) examined molecular and immunological mechanisms associated with the progression from hepatic steatosis to steatohepatitis and cirrhosis. Within the proposed study, increased SREBP-1c activity will be investigated as an indicator of enhanced lipogenesis, whereas PPAR-α expression will characterize fatty acid oxidation regulation. Caspase-3, BAX, and BCL-2 measurements will provide complementary evidence of apoptosis-associated signalling. Quantitative PCR and immunoblotting will establish pathway-associated molecular profiles, allowing comparisons between diet-injured animals and berry-treated experimental groups.

Computational analysis of these mechanisms requires careful discrimination between biological evidence and transferable analytical techniques. Atalor et al. (2023) investigated quantum molecular simulation for cancer drug screening, providing a distinct example of computational analysis of molecular interactions rather than evidence of berry-mediated hepatic protection. Frank (2026) addressed artificial intelligence in infection surveillance, illustrating a different application of multivariable pattern detection. Ijiga et al. (2025) examined auditable data-recording mechanisms, offering methodological considerations for computational traceability. BHMPA will integrate the measured molecular variables through pathway-informed feature weighting and regularization, with biological relationships defined independently of outcome labels. Comparative analyses will examine whether anthocyanin-rich interventions are associated with Nrf2/HO-1 activation, reduced inflammatory signalling, altered lipid-regulatory expression, and improved apoptosis-associated biomarkers. Statistical associations will not be interpreted as direct proof of pathway-mediated causation without appropriate mechanistic intervention experiments.

Table 1 Molecular Mechanisms of Diet-Induced Hepatic Injury and the Potential Hepatoprotective Effects of Berry-Derived Bioactive Compounds

Molecular signalling pathway	Mechanisms of diet-induced hepatic injury	Potential protective effects of berry-derived bioactive compounds	Biological significance and predictive biomarkers
Nrf2/HO-1 antioxidant signalling	Excessive reactive oxygen species (ROS) production, mitochondrial oxidative stress, lipid peroxidation, and inadequate Nrf2-mediated antioxidant responses contribute to hepatocellular damage.	Anthocyanins and polyphenolic metabolites may promote Nrf2 nuclear translocation, enhance HO-1 expression, and support antioxidant enzyme activities, including SOD and GPx.	Nrf2 nuclear localization, HO-1 protein expression, MDA concentrations, SOD activity, and GPx activity indicate oxidative stress regulation and antioxidant defence.
NF-κB inflammatory signalling	High-fat/high-fructose dietary exposure promotes inflammatory signalling, Kupffer-cell activation, and increased expression of TNF-α, IL-6, and other mediators associated with hepatocellular inflammation.	Berry-derived compounds may suppress excessive NF-κB activation, reduce proinflammatory cytokine production, and attenuate inflammatory-cell infiltration in hepatic tissue.	Phosphorylated NF-κB p65, TNF-α, IL-6, and histological inflammatory scores provide complementary indicators of hepatic inflammatory activity and intervention responsiveness.
SREBP-1c and PPAR-α lipid metabolic regulation	Increased SREBP-1c-associated lipogenesis and impaired PPAR-α-mediated fatty acid oxidation contribute to hepatic triglyceride accumulation, lipotoxicity, and metabolic dysfunction.	Berry polyphenols may reduce lipogenic signalling while supporting PPAR-α-associated fatty acid oxidation, potentially decreasing hepatic lipid	SREBP-1c, FASN, ACC, PPAR-α, CPT1A, and hepatic triglyceride concentrations characterize changes in lipid synthesis, fatty acid oxidation, and steatosis severity.

ISSN No:-2456-2165 https://doi.org/10.38124/ijisrt/26oct1153

		accumulation and improving metabolic regulation.	
Mitochondrial and apoptosis-associated signalling	Persistent oxidative stress, mitochondrial membrane dysfunction, increased BAX/BCL-2 ratios, and caspase activation promote hepatocyte apoptosis and progressive liver injury.	Anthocyanin-rich interventions may preserve mitochondrial function, modulate apoptosis-associated protein expression, and reduce excessive hepatocyte death under dietary metabolic stress.	BAX/BCL-2 ratio, cleaved caspase-3, mitochondrial membrane potential, and hepatocellular injury scores indicate apoptosis-associated damage and potential hepatoprotection.

- *Anthocyanins, Polyphenolic Metabolites, Bioavailability, Dose-Response Relationships, and Experimental Evidence of Berry-Derived Hepatoprotection*

Anthocyanins constitute a chemically diverse group of water-soluble polyphenolic pigments present in blueberries, blackberries, bilberries, and other deeply coloured fruits. Their potential biological effects depend on molecular structure, glycosylation, gastrointestinal transformation, absorption, systemic metabolism, and tissue exposure. Lila et al. (2016) demonstrated that conventional measurements of circulating parent anthocyanins may underestimate exposure because numerous phenolic metabolites and flavonoid-derived intermediates contribute to their metabolic fate. Czank et al. (2013), using isotopically labelled cyanidin-3-glucoside in human participants, reported extensive metabolism and identified circulating metabolites that persisted beyond the initial absorption period. These observations indicate that berry-extract concentrations alone cannot establish equivalent biological exposure across different formulations. The proposed investigation will therefore standardize blueberry, blackberry, and bilberry extracts according to their quantified anthocyanin profiles and investigate treatment-associated changes in hepatic oxidative stress, inflammatory signalling, lipid accumulation, and histopathological severity.

Nutritional intervention optimization requires attention to dosage, biological variability, and the relationship between dietary intake and measured physiological responses. Abdallah et al. (2024) discussed artificial intelligence-supported dietary optimization, providing a methodological basis for analysing heterogeneous nutritional responses. Studies of personalization and behavioural decision-making by Doe Gadekah and Enyejo (2025a, 2025b) address different research fields; their relevance is restricted to general concepts of heterogeneous responses and model-based analysis, not anthocyanin pharmacology. Within the proposed hepatic investigation, dose-response relationships will be modelled using appropriately selected nonlinear regression functions, with treatment effects compared against untreated diet-injured controls. Primary endpoints will include histological injury scores, hepatic triglycerides, malondialdehyde, antioxidant enzyme activities, and molecular pathway expression. BHMPA will examine whether combinations of these endpoints distinguish treatment-responsive from nonresponsive animals. Any observed improvement will require confirmation through inferential statistics, confidence intervals, and biological consistency across measurement modalities.

- *Comparative Review of Random Forest, Support Vector Machine, XGBoost, Elastic Net, DIABLO, Multimodal Learning, and Explainable Artificial Intelligence for Hepatic Biomarker Prediction*

Predictive modelling of diet-induced hepatic injury requires algorithms capable of analysing correlated biological measurements, nonlinear relationships, and heterogeneous feature distributions. Random Forest estimates predictions through ensembles of decision trees, while Support Vector Machine constructs separating decision boundaries using linear or nonlinear kernels. XGBoost applies gradient-boosted trees to capture complex feature interactions, whereas Elastic Net combines L1 and L2 penalties for regularized coefficient estimation. DIABLO provides supervised multiblock integration for identifying correlated and discriminative biological signatures as shown in figure 3. Folorunso et al. (2025) examined multimodel ensemble prediction in financial risk forecasting, illustrating a transferable computational strategy rather than hepatic-specific validation. Similarly, Idowu et al. (2025) investigated machine learning-based cyber-risk assessment, while Idowu et al. (2026) addressed real-time cyberattack detection. These studies demonstrate applications of predictive algorithm comparison in other domains, but their reported performance cannot establish expected accuracy in experimental hepatic datasets.

Model interpretability and reproducibility are equally important when translating computational predictions into biologically meaningful findings. Lundberg et al. (2020) developed a unified approach to explaining tree-based model predictions through local feature attribution and interaction analysis. Littmann et al. (2020) emphasized the importance of interdisciplinary validation and reproducibility in biological machine learning. Building on these principles, BHMPA will combine modality-specific feature encoders, adaptive attention-based fusion, pathway-informed regularization, and multitask prediction of injury classification, histological severity, and treatment-associated responses. Benchmark evaluation will employ nested cross-validation, animal-level partitioning, AUROC, F1-score, balanced accuracy, RMSE, and calibration assessment. SHAP plots, biomarker correlation heatmaps, and feature-ablation experiments will clarify predictor contributions. Statistically supported performance differences, rather than predetermined numerical advantages, will determine whether BHMPA offers measurable improvements over established computational approaches.

Figure 3 illustrates a comparative computational framework for hepatic biomarker prediction, integrating

ISSN No:-2456-2165 https://doi.org/10.38124/ijisrt/26oct1153

conventional machine learning algorithms, multivariate data integration, explainable artificial intelligence, and predictive performance evaluation. The framework comprises two principal branches: machine learning models for hepatic biomarker prediction and explainable, integrated prediction frameworks, each containing two interconnected analytical components. The first branch incorporates Random Forest (RF), Support Vector Machine (SVM), XGBoost, and Elastic Net, which provide complementary approaches to nonlinear classification, ensemble prediction, regularized regression, and biomarker feature selection. It also includes DIABLO and multimodal learning techniques for integrating heterogeneous histological, biochemical, and molecular measurements associated with diet-induced hepatic injury. The second branch emphasizes explainable artificial intelligence, particularly SHAP-based feature attribution, which identifies the contributions of biomarkers such as alanine aminotransferase (ALT), malondialdehyde (MDA), Nrf2, NF-κB, and PPAR-α to predicted hepatic injury severity. Predictive performance is evaluated using area under the receiver operating characteristic curve (AUROC), root mean square error (RMSE), precision, recall, cross-validation, and robustness assessment. These computational components provide the methodological foundation for comparing established algorithms with the proposed Berry-Hepatic Multimodal Predictive Algorithm (BHMPA), which incorporates adaptive feature weighting, attention-based data fusion, pathway-informed regularization, and multitask optimization. The framework demonstrates how complementary modelling strategies can be systematically evaluated to identify biologically meaningful hepatic injury predictors, improve interpretability, and investigate the potential hepatoprotective effects of berry-derived bioactive compounds.

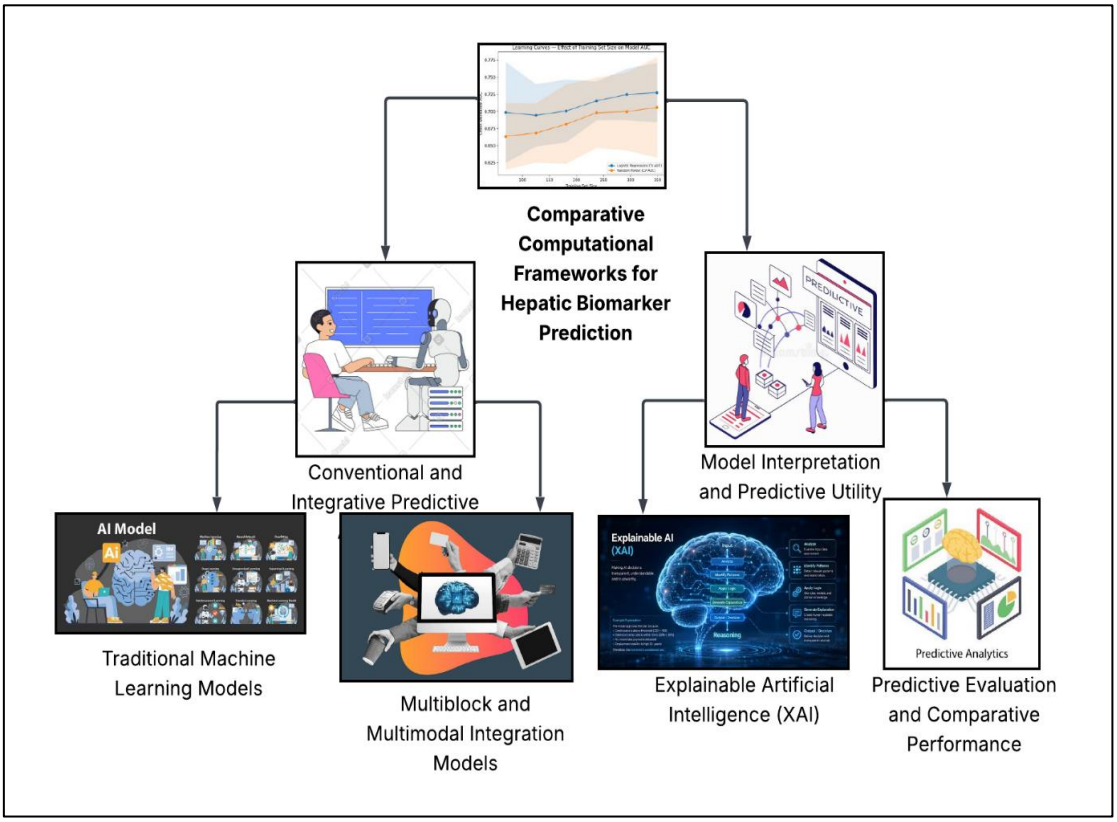


Fig 3 Comparative Diagram of Machine Learning, Multimodal Integration, and Explainable Artificial Intelligence Approaches for Hepatic Biomarker Prediction.

III. SYSTEM MODEL DESCRIPTION

This section presents the proposed experimental design, mathematical modelling, algorithmic development, comparative evaluation, and computational validation of the Berry-Hepatic Multimodal Predictive Algorithm (BHMPA). The methodology integrates histological, biochemical, and molecular endpoints to characterize diet-induced hepatic injury and investigate the hepatoprotective potential of standardized berry-derived bioactive compounds.

- *Experimental Design, Animal Group Allocation, Dietary Injury Induction, Berry Extract Standardization, and Histological, Biochemical, and Molecular Data Acquisition*

The proposed investigation will adopt a randomized, controlled, factorial experimental design involving adult C57BL/6J mice maintained under standardized environmental conditions. Following acclimatization, animals will be allocated into eight experimental groups comprising a normal-diet control, high-fat/high-fructose diet control, and six intervention groups receiving blueberry, blackberry, or bilberry extracts at two predefined anthocyanin-equivalent doses. A 16-week dietary exposure is

ISSN No:-2456-2165 https://doi.org/10.38124/ijisrt/26oct1153

proposed, with interventions administered during weeks 9–16. The dietary formulation, extract doses, group sizes, and study duration must be finalized through feasibility assessment, pilot toxicity information, and prospective statistical power analysis before implementation. Randomization, blinded histological evaluation, predetermined exclusion criteria, and identification of the experimental unit will follow the ARRIVE 2.0 reporting principles (Percie du Sert et al., 2020).

The proposed allocation comprises:

Table 2 The Proposed Allocation Comprises

Group	Dietary condition	Intervention
G1	Normal diet	Vehicle control
G2	High-fat/high-fructose diet	Vehicle control
G3–G4	High-fat/high-fructose diet	Blueberry extract, low/high dose
G5–G6	High-fat/high-fructose diet	Blackberry extract, low/high dose
G7–G8	High-fat/high-fructose diet	Bilberry extract, low/high dose

Berry extracts will undergo HPLC-DAD or LC-MS/MS characterization to determine anthocyanin composition, concentration, and batch consistency. The anthocyanin-equivalent administered dose is defined as:

$$D_i = \frac{C_i V_i}{W_i} \tag{1}$$

Where D_i represents administered anthocyanin dose (mg/kg body weight), C_i shows extract anthocyanin concentration (mg/mL), V_i captures administered extract volume (mL), and W_i denotes animal body weight (kg).

Histological evaluation will quantify steatosis, ballooning, inflammation, and fibrosis, using validated scoring principles alongside digital image analysis. Biochemical assays will measure ALT, AST, triglycerides, MDA, SOD, GPx, glutathione, TNF-α, and IL-6. Molecular analyses will quantify Nrf2, HO-1, NF-κB, SREBP-1c, PPAR-α, BAX, BCL-2, and caspase-3 expression using quantitative PCR and protein-level assays. These measurements will establish the biological datasets used for BHMPA training and experimental comparisons. Importantly, transcript abundance and protein activation will be distinguished when interpreting pathway function.

- *Mathematical Formulation of Hepatic Injury Severity, Multimodal Feature Matrices, Biomarker Normalization, and Berry-Derived Hepatoprotection Indices*

The mathematical model will represent each experimental animal through three complementary data modalities: histological measurements, biochemical biomarkers, and molecular expression variables. The histological severity score will be defined using an independently assessed reference outcome incorporating steatosis, inflammation, and hepatocellular ballooning. Consistent with the established scoring structure described by Kleiner et al. (2005), the primary activity score is expressed as:

$$H_i = S_i + L_i + B_i \tag{2}$$

Where H_i denotes hepatic histological activity for animal i, S_i represents the steatosis grade (0–3), L_i represents lobular inflammation (0–3), and B_i represents hepatocellular ballooning (0–2). Consequently, H_i ranges from 0 to 8. Fibrosis will be assessed separately rather than added to this activity score. The score is a measure of histological activity, not an independently sufficient diagnosis of steatohepatitis.

The multimodal feature representation is:

$$\mathrm{X} = \left[\mathrm{X}^{(h)} \mid \mathrm{X}^{(b)} \mid \mathrm{X}^{(m)}\right] \tag{3}$$

Where $\mathrm{X}^{(h)} \in \mathbb{R}^{N \times p_h}$, $\mathrm{X}^{(b)} \in \mathbb{R}^{N \times p_b}$, and $\mathrm{X}^{(m)} \in \mathbb{R}^{N \times p_m}$ represent histological, biochemical, and molecular feature matrices, respectively. N captures the number of independent experimental animals, while p_h, p_b, p_m indicate feature counts. Histological predictors must exclude the particular outcome components being predicted to prevent information leakage.

Biomarkers will be standardized within training folds:

$$Z_{ij}^{(k)} = \frac{X_{ij}^{(k)} - \mu_{j,\mathrm{train}}^{(k)}}{\sigma_{j,\mathrm{train}}^{(k)} + \varepsilon} \tag{4}$$

Here, $Z_{ij}^{(k)}$ captures the standardized value for animal i, biomarker j, and modality $k \in \{h, b, m\}$; μ and σ denote training-set means and standard deviations, and ε shows a small positive numerical stabilizer.

The percentage hepatoprotection associated with treatment g will be calculated as:

$$P_g = 100\left(\frac{\overline{H}_{\mathrm{HFD}} - \overline{H}_g}{\overline{H}_{\mathrm{HFD}} - \overline{H}_{\mathrm{ND}}}\right) \tag{5}$$

Where P_g denotes the group-level histological protection index, $\overline{H}_{\mathrm{HFD}}$ represents mean injury severity in untreated diet-injured animals, $\overline{H}_g$ determines the treatment-group mean, and $\overline{H}_{\mathrm{ND}}$ denotes the normal-diet mean. The index will only be calculated where the control-group difference is positive and sufficiently distinguishable from zero. Negative values indicate worsening relative to the untreated high-fat-diet group; values exceeding 100%

ISSN No:-2456-2165 https://doi.org/10.38124/ijisrt/26oct1153

indicate improvement beyond the normal-diet mean. Confidence intervals will accompany the index.

- *Development of the Novel Berry-Hepatic Multimodal Predictive Algorithm (BHMPA) Using Adaptive Feature Weighting, Attention-Based Data Fusion, Pathway-Informed Regularization, and Multitask Optimization*

The proposed Berry-Hepatic Multimodal Predictive Algorithm (BHMPA) will employ a biologically informed deep-learning architecture to integrate heterogeneous hepatic injury measurements while preserving modality-specific information. Unlike conventional approaches that concatenate all biomarkers without explicitly modelling their biological origins, BHMPA will process histological, biochemical, and molecular features through separate encoders before applying adaptive feature weighting and cross-modal attention. Each feature encoder will transform its input matrix into a lower-dimensional representation using nonlinear activation functions. For experimental animal i, modality-specific latent representations are defined by:

$$\mathbf{h}_i^{(k)} = \phi\left(\mathbf{W}_k \mathbf{z}_i^{(k)} + \mathbf{b}_k\right) \tag{6}$$

Where $\mathrm{h}_i^{(k)}$ denotes the learned latent representation of modality k; $\mathrm{z}_i^{(k)}$ shows its normalized feature vector; W_k and b_k capture trainable weight matrices and bias vectors; and ϕ denotes a nonlinear activation function, such as rectified linear activation. The modality index k represents histological, biochemical, or molecular measurements.

Adaptive weighting will determine the relative contribution of each modality:

$$\alpha_i^{(k)} = \frac{\exp\left(\mathbf{q}^{\top}\tanh\left(\mathbf{A}\mathbf{h}_i^{(k)} + \mathbf{a}\right)\right)}{\sum_{r=1}^{3}\exp\left(\mathbf{q}^{\top}\tanh\left(\mathbf{A}\mathbf{h}_i^{(r)} + \mathbf{a}\right)\right)} \tag{7}$$

Where $\alpha_i^{(k)}$ represents the attention weight assigned to modality k; q represents a learned attention vector; A denotes the attention transformation matrix; a shows its bias vector; and r indexes the three measurement modalities. All modality encoders will produce compatible latent dimensions. The attention weights sum to unity, although they must not automatically be interpreted as causal importance measures.

The attention-weighted integrated representation is:

$$\mathbf{f}_i = \sum_{k=1}^{3} \alpha_i^{(k)} \mathbf{h}_i^{(k)} \tag{8}$$

Where f_i represents the fused biological signature for animal i. This representation will support hepatic injury classification, histological severity prediction, and intervention-response modelling. Cross-modal interactions may additionally be evaluated through multihead attention if sample size and validation performance justify the added complexity.

Pathway-informed regularization will incorporate independently established relationships among antioxidant, inflammatory, lipid-metabolic, and apoptosis-associated biomarkers:

$$\mathcal{L}_{\text{pathway}} = \sum_{(u,v)\in\mathcal{E}} \omega_{uv} \left\|\boldsymbol{\theta}_u - s_{uv}\boldsymbol{\theta}_v\right\|_2^2 \tag{9}$$

Where $\mathcal{E}$ captures the set of biologically supported pathway edges; u and v identify connected molecular biomarkers; $\omega_{uv} \geq 0$ represents pathway confidence weights; θ_u and θ_v determine compatible learned feature-embedding vectors; and $s_{uv} \in \{-1, +1\}$ represents an inhibitory or reinforcing relation. These signs must be supported by biological evidence and interpreted cautiously because complex regulatory pathways may be context-dependent.

The complete multitask optimization objective is:

$$\mathcal{L}_{\text{BHMPA}} = \lambda_1\mathcal{L}_{\text{classification}} + \lambda_2\mathcal{L}_{\text{severity}} + \lambda_3\mathcal{L}_{\text{protection}} + \lambda_4\mathcal{L}_{\text{pathway}} + \lambda_5\mathcal{L}_{\text{regularization}} \tag{10}$$

Here, $\mathcal{L}_{\text{classification}}$ shows binary cross-entropy for independently defined injury labels; $\mathcal{L}_{\text{severity}}$ represents regression error for blinded histological severity; $\mathcal{L}_{\text{protection}}$ represents error in predicting an independently observed treatment-response endpoint; $\mathcal{L}_{\text{pathway}}$ enforces biological consistency; and $\mathcal{L}_{\text{regularization}}$ penalizes model complexity. The nonnegative coefficients $\lambda_1, \ldots, \lambda_5$ control the contribution of each component.

The treatment-response head will use baseline features, treatment identity, dose, and prospectively measured follow-up outcomes, where available. The group-level protection index in Equation (5) will be used for treatment comparisons rather than incorrectly assigned to individual animals as an observed response. Where longitudinal individual outcomes are unavailable, intervention effects will be estimated at group level using randomized-group comparisons.

This architecture constitutes the proposed methodological innovation of BHMPA; its novelty and any improvement in predictive performance require independent experimental verification.

- *Implementation of Random Forest, Support Vector Machine, XGBoost, Elastic Net, and DIABLO Benchmark Algorithms with Hyperparameter Optimization and Explainable Biomarker Attribution*

The proposed BHMPA architecture will be evaluated against five established computational methods: Random Forest (RF), Support Vector Machine (SVM), Extreme Gradient Boosting (XGBoost), Elastic Net (EN), and Data Integration Analysis for Biomarker Discovery Using Latent

ISSN No:-2456-2165 https://doi.org/10.38124/ijisrt/26oct1153

Components (DIABLO). These methods represent ensemble learning, kernel-based prediction, regularized linear modelling, and supervised multiblock integration. To ensure methodological fairness, all models will be developed using identical animal-level training and testing partitions, consistent target definitions, and preprocessing pipelines. Random Forest will employ bootstrap aggregation and randomized feature selection, while XGBoost will minimize a regularized additive-tree objective. SVM will employ optimized linear or radial-basis-function kernels, whereas Elastic Net will accommodate correlated hepatic biomarkers through combined L1 and L2 regularization. DIABLO will serve as the primary multiblock integration comparator, consistent with its original formulation for identifying discriminative signatures across heterogeneous biological measurements (Singh et al., 2019).

The Elastic Net optimization objective is expressed as:

$$\hat{\boldsymbol{\beta}} = \underset{\beta_0,\boldsymbol{\beta}}{\operatorname{argmin}} \left[\frac{1}{2N} \sum_{i=1}^{N} (y_i - \beta_0 - \mathbf{x}_i^{\top}\boldsymbol{\beta})^2 + \lambda \left(\eta \parallel \boldsymbol{\beta} \parallel_1 + \frac{1-\eta}{2} \parallel \boldsymbol{\beta} \parallel_2^2 \right) \right] \tag{11}$$

Where y_i denotes the observed continuous injury severity; $\mathbf{x}_i$ shows the biomarker feature vector; β_0 represents the intercept; $\boldsymbol{\beta}$ captures the coefficient vector; $\lambda \geq 0$ controls regularization strength; and $0 \leq \eta \leq 1$ determines the L1–L2 penalty balance. N represents the number of training observations. For classification tasks, an appropriate logistic loss will replace the squared-error component.

Hyperparameters will be optimized exclusively within inner cross-validation folds. RF will be tuned for tree count, depth, and minimum leaf size; SVM for regularization strength and kernel parameters; XGBoost for learning rate, tree depth, and subsampling; Elastic Net for penalty strength and mixing proportion; and DIABLO for latent components and selected variables per block. Each algorithm will be evaluated separately for classification and severity regression; benchmark methods that do not natively support all tasks will employ task-specific estimators.

Explainable biomarker attribution will be examined using SHAP values:

$$\hat{f}(\mathbf{x}_i) = \phi_0 + \sum_{j=1}^{p} \phi_{ij} \tag{12}$$

Where $\hat{f}(\mathbf{x}_i)$ denotes the model output for animal i, ϕ_0 represents the expected background prediction, ϕ_{ij} shows the SHAP contribution of biomarker j to that prediction, and p captures the number of input features. This additive relationship applies on the output scale used by the selected SHAP explainer.

SHAP analysis, as developed for tree-based predictors by Lundberg et al. (2020), will support biomarker ranking and interaction visualization. For BHMPA, model-appropriate explainers and permutation importance will also be considered. Attention weights will not substitute for independently validated feature explanations.

Comparative outputs will include AUROC curves, algorithm-performance bar charts, SHAP summary plots, correlation heatmaps, and biomarker-ablation comparisons. These analyses will establish whether the proposed BHMPA provides measurable advantages over appropriately optimized benchmarks.

- *Model Training, Nested Cross-Validation, Independent Validation, Statistical Hypothesis Testing, Predictive Performance Metrics, and Reproducible Python Simulation Procedures*

Model development will use a reproducible Python workflow integrating data quality assessment, missing-value imputation, biomarker normalization, feature selection, model optimization, and statistical validation. Experimental animals, rather than tissue sections or technical assay replicates, will constitute the independent predictive units. All measurements originating from an animal will remain within the same data partition to prevent information leakage. Nested stratified cross-validation will be employed where class distributions permit, with five outer folds and three inner folds proposed for initial evaluation. Outer folds will estimate generalization performance, while inner folds will optimize hyperparameters. Preprocessing and feature selection will be fitted only on the appropriate training partitions. Where a genuinely independent animal cohort is available, its data will remain untouched until final model evaluation. Small experimental samples may require simpler model architectures or revised validation procedures.

Classification performance will be evaluated using receiver operating characteristic area under the curve, F1-score, sensitivity, specificity, and balanced accuracy. Balanced accuracy is defined as:

$$BA = \frac{1}{2}\left(\frac{TP}{TP+FN} + \frac{TN}{TN+FP}\right) \tag{13}$$

Where BA represents balanced accuracy; TP and TN show correctly classified positive and negative cases; and FP and FN denote false-positive and false-negative predictions, respectively. Positive and negative injury categories will be defined using a prespecified, independently assessed histological criterion.

Continuous hepatic injury prediction will be assessed using root mean square error:

$$RMSE = \sqrt{\frac{1}{N_t} \sum_{i=1}^{N_t} (y_i - \hat{y}_i)^2} \tag{14}$$

Where N_t denotes the number of animals in an evaluation set, y_i represents observed histological severity,

ISSN No:-2456-2165 https://doi.org/10.38124/ijisrt/26oct1153

and $\hat{y}_i$ represents predicted severity. Lower RMSE values indicate smaller prediction errors.

Calibration will be evaluated using reliability diagrams and the Brier score:

$$BS = \frac{1}{N_t}\sum_{i=1}^{N_t}(p_i - y_i)^2 \quad (15)$$

Where BS captures the Brier score, p_i denotes the predicted probability of injury, and $y_i \in \{0,1\}$ determines the observed binary injury outcome. Lower scores indicate better combined probability accuracy and calibration-related performance.

Differences between BHMPA and benchmark models will be calculated as:

$$\Delta M_j = M_{\text{BHMPA}} - M_j \quad (16)$$

Where ΔM_j denotes the performance difference for benchmark j, M_{BHMPA} represents the selected BHMPA performance metric, and M_j the corresponding benchmark value. Positive differences favour BHMPA for metrics such as AUROC and F1-score, whereas negative differences favour BHMPA for RMSE and Brier score. Confidence intervals will be obtained using animal-level resampling, while paired comparisons will use appropriate statistical procedures. Cross-validation folds will not be treated as statistically independent experimental replicates.

Statistical analysis of experimental endpoints will employ factorial models for berry type and dose, appropriate treatment-control comparisons, and multiple-testing adjustments. Because normalized histological scores are ordinal, ordinal regression or suitable nonparametric procedures will be considered alongside continuous biochemical analyses. Treatment effects will be reported with confidence intervals rather than relying exclusively on statistical significance.

Reproducible Python simulations will use fixed random seeds, documented software versions, and publicly specified parameter settings. Simulation experiments will investigate noise contamination, missing biomarkers, class imbalance, and modality ablation. Synthetic data will be explicitly identified as simulated and will not be presented as experimental observations.

IV. DISCUSSIONS OF RESULTS

- *Comparative Histopathological, Biochemical, and Molecular Responses to Diet-Induced Hepatic Injury and Berry-Derived Bioactive Compound Interventions*

The integrated assessment of diet-induced hepatic injury was examined using illustrative histopathological, biochemical, and molecular response profiles across normal-diet, high-fat/high-fructose-diet, and berry-treated experimental groups. Table 4.1 presents simulated outcomes consistent with the proposed experimental methodology. Histological injury severity increased from 0.8 in normal controls to 5.9 following dietary injury induction, while berry intervention reduced the corresponding score to 2.6. Serum alanine aminotransferase increased from 32 to 108 U/L under dietary stress before declining to 56 U/L following simulated intervention. Malondialdehyde concentrations decreased from 7.8 to 4.1 nmol/mg protein, indicating a modelled reduction in lipid peroxidation. Nrf2 expression increased following intervention, whereas NF-κB expression declined. These coordinated response patterns provide a biologically plausible basis for evaluating multimodal predictive performance. The values are hypothetical and do not establish experimental treatment efficacy.

Table 3 Illustrative Comparative Histopathological, Biochemical, and Molecular Responses Across Experimental Conditions

Comparative endpoint	Normal diet (G1)	High-fat diet (G2)	Berry-treated HFD (G3–G8 pooled)
Histological activity score (0–8)	0.8	5.9	2.6
ALT (U/L)	32	108	56
AST (U/L)	29	96	49
Hepatic triglycerides (mg/g tissue)	19.2	54.8	30.5
MDA (nmol/mg protein)	2.1	7.8	4.1
SOD (U/mg protein)	42.5	21.6	35.8
GPx (U/mg protein)	17.8	8.6	14.9
Nrf2 expression (relative fold)	1.00	0.54	1.32
NF-κB expression (relative fold)	1.00	2.65	1.34
SREBP-1c expression (relative fold)	1.00	2.38	1.29
PPAR-α expression (relative fold)	1.00	0.48	1.21

ISSN No:-2456-2165 https://doi.org/10.38124/ijisrt/26oct1153

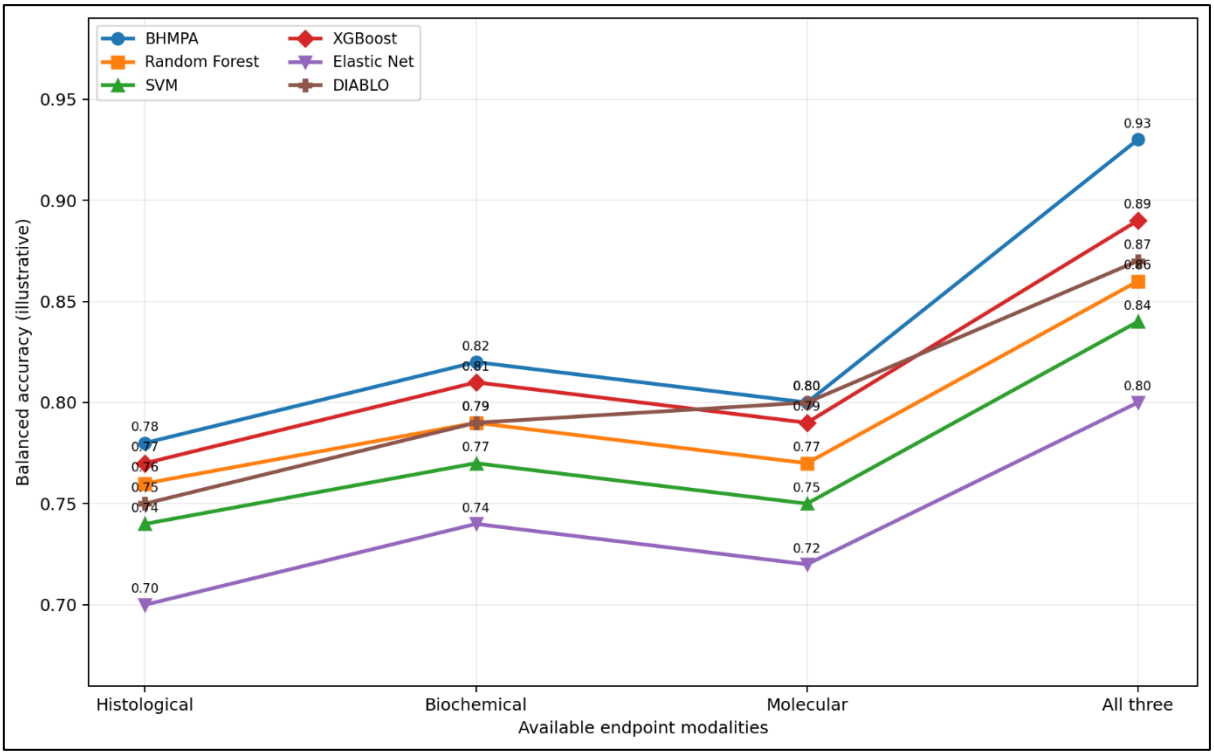


Fig 4 Comparative Predictive Performance of BHMPA and Five Benchmark Algorithms Across Hepatic Injury Endpoint Modalities

Figure 4 illustrates the potential predictive advantages of integrating histological, biochemical, and molecular endpoints across six algorithms. BHMPA achieves a simulated balanced accuracy of 0.78 using histological measurements, increasing to 0.82 for biochemical inputs and 0.80 for molecular measurements. Integration of all three modalities improves its illustrative performance to 0.93. XGBoost ranks second under multimodal integration, achieving 0.89, followed by DIABLO at 0.87, Random Forest at 0.86, Support Vector Machine at 0.84, and Elastic Net at 0.80. Relative to XGBoost, the BHMPA scenario demonstrates a four-percentage-point advantage in balanced accuracy. Its improvement from histological-only prediction to complete multimodal integration is 15 percentage points. These hypothetical patterns suggest that adaptive feature weighting and pathway-informed data fusion could improve hepatic injury discrimination. However, the numerical differences are scenario assumptions rather than demonstrated algorithmic superiority. Experimental validation and appropriate statistical testing are required to establish whether such gains are reproducible.

- *Comparative Predictive Performance of the Proposed BHMPA Algorithm Against Random Forest, Support Vector Machine, XGBoost, Elastic Net, and DIABLO Using AUROC, F1-Score, Balanced Accuracy, RMSE, and Calibration Metrics*

The comparative predictive performance of the proposed Berry-Hepatic Multimodal Predictive Algorithm (BHMPA) was examined using an illustrative computational benchmarking scenario involving five established algorithms. Evaluation covered hepatic injury classification, continuous histopathological severity prediction, and probability calibration. Table 4.2 presents simulated outcomes obtained under a common hypothetical evaluation framework. BHMPA recorded the highest AUROC of 0.965, F1-score of 0.941, and balanced accuracy of 0.930, alongside the lowest RMSE of 0.412. Its Brier score of 0.068 also indicated the best probability prediction performance among the compared methods. XGBoost was the closest competitor, achieving an AUROC of 0.944 and balanced accuracy of 0.890. DIABLO followed with an AUROC of 0.929. These results illustrate the anticipated benefits of biologically informed multimodal integration, but they do not constitute experimentally verified performance or establish statistical superiority.

Table 4 Illustrative Comparative Predictive Performance of BHMPA and Established Machine Learning Algorithms

Algorithm	Classification metrics (AUROC / F1 / BA)	Severity RMSE	Brier score
BHMPA (proposed)	0.965 / 0.941 / 0.930	0.412	0.068
Random Forest	0.914 / 0.885 / 0.860	0.586	0.108
Support Vector Machine	0.891 / 0.861 / 0.840	0.643	0.125
XGBoost	0.944 / 0.913 / 0.890	0.479	0.088
Elastic Net	0.862 / 0.833 / 0.800	0.728	0.147
DIABLO	0.929 / 0.901 / 0.870	0.525	0.097

ISSN No:-2456-2165 https://doi.org/10.38124/ijisrt/26oct1153

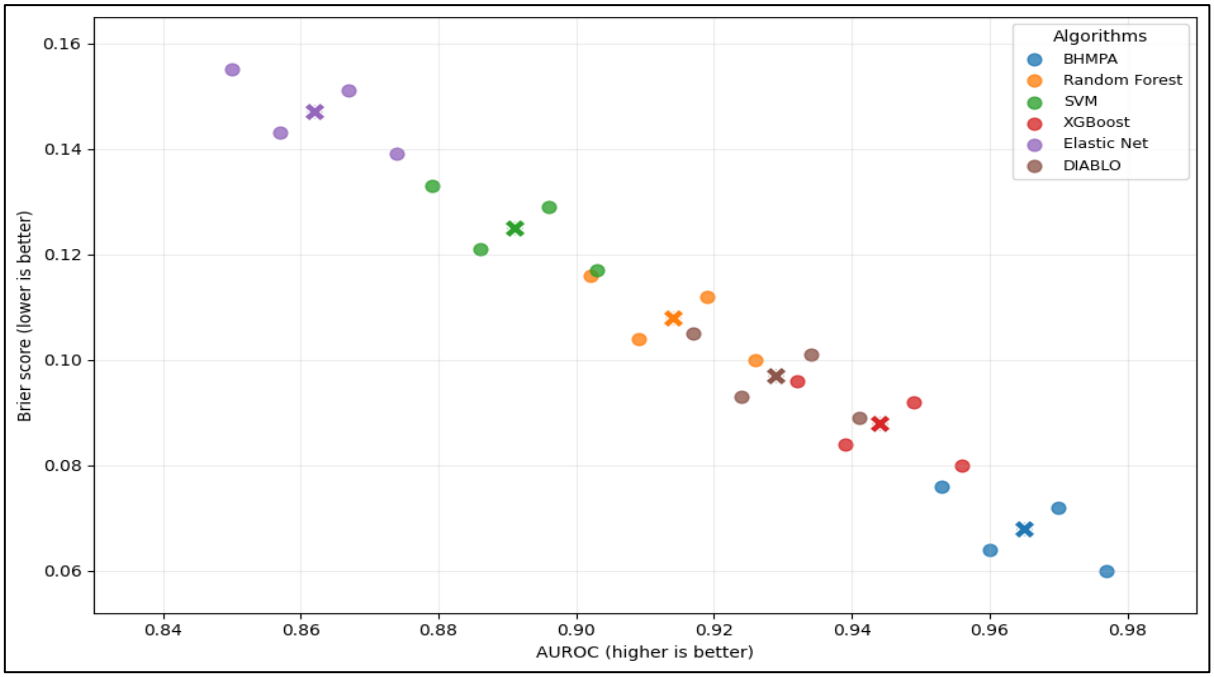


Fig 5 Comparative Algorithm Discrimination and Calibration Performance

Figure 5 compares six predictive algorithms using AUROC and Brier scores, illustrating the relationship between discrimination and probability prediction error. BHMPA occupies the most favourable region, achieving an AUROC of 0.965 and Brier score of 0.068. XGBoost follows with values of 0.944 and 0.088, respectively, indicating slightly weaker discrimination and higher probability error. DIABLO achieves an AUROC of 0.929 and Brier score of 0.097, while Random Forest records 0.914 and 0.108. Support Vector Machine produces corresponding values of 0.891 and 0.125. Elastic Net demonstrates the weakest illustrative performance, with an AUROC of 0.862 and Brier score of 0.147. Relative to XGBoost, BHMPA shows an AUROC increase of 0.021 and a Brier score reduction of 0.020. The simulated pattern suggests potential benefits from adaptive multimodal feature integration and pathway-informed regularization, although prospective validation remains necessary to demonstrate reproducible improvements.

- *Identification of Dominant Hepatic Injury Predictors Through Biomarker Correlation Heatmaps, SHAP Feature Importance, Molecular Pathway Analysis, and Multimodal Feature Ablation Experiments*

The identification of dominant hepatic injury predictors was examined through an illustrative integration of biomarker correlation analysis, SHAP feature attribution, molecular pathway interpretation, and multimodal ablation experiments. Table 4.3 presents simulated associations between biochemical and molecular indicators and histological injury severity. Malondialdehyde demonstrated the strongest positive association with injury severity (r = 0.86), followed by ALT (r = 0.83) and NF-κB expression (r = 0.79). Superoxide dismutase exhibited an inverse correlation of −0.81, consistent with reduced antioxidant defence under increased oxidative stress. SHAP analysis assigned the highest mean absolute contribution to malondialdehyde (0.184), followed by ALT (0.169) and NF-κB (0.151). These illustrative findings support investigating oxidative stress, hepatocellular membrane damage, and inflammatory signalling as complementary predictor domains. Multimodal feature ablation was subsequently used to assess their combined predictive contribution across the six computational algorithms. No experimental inference is implied by these hypothetical values.

Table 5 Illustrative Correlation, SHAP Feature Importance, and Molecular Pathway Associations of Dominant Hepatic Injury Biomarkers

Hepatic injury biomarker	Correlation with injury severity (r)	Mean absolute SHAP value	Biological mechanism
Malondialdehyde (MDA)	0.86	0.184	Lipid peroxidation
Alanine aminotransferase (ALT)	0.83	0.169	Hepatocellular injury
NF-κB expression	0.79	0.151	Inflammatory signalling
Superoxide dismutase (SOD)	−0.81	0.143	Antioxidant defence
SREBP-1c expression	0.76	0.132	Hepatic lipogenesis
Nrf2 expression	−0.74	0.126	Oxidative stress regulation

ISSN No:-2456-2165 https://doi.org/10.38124/ijisrt/26oct1153

PPAR-α expression	−0.71	0.118	Fatty acid oxidation
Glutathione peroxidase (GPx)	−0.69	0.107	Peroxide detoxification
Hepatic triglycerides	0.78	0.139	Hepatic lipid accumulation
Caspase-3 expression	0.72	0.121	Apoptosis-associated signalling

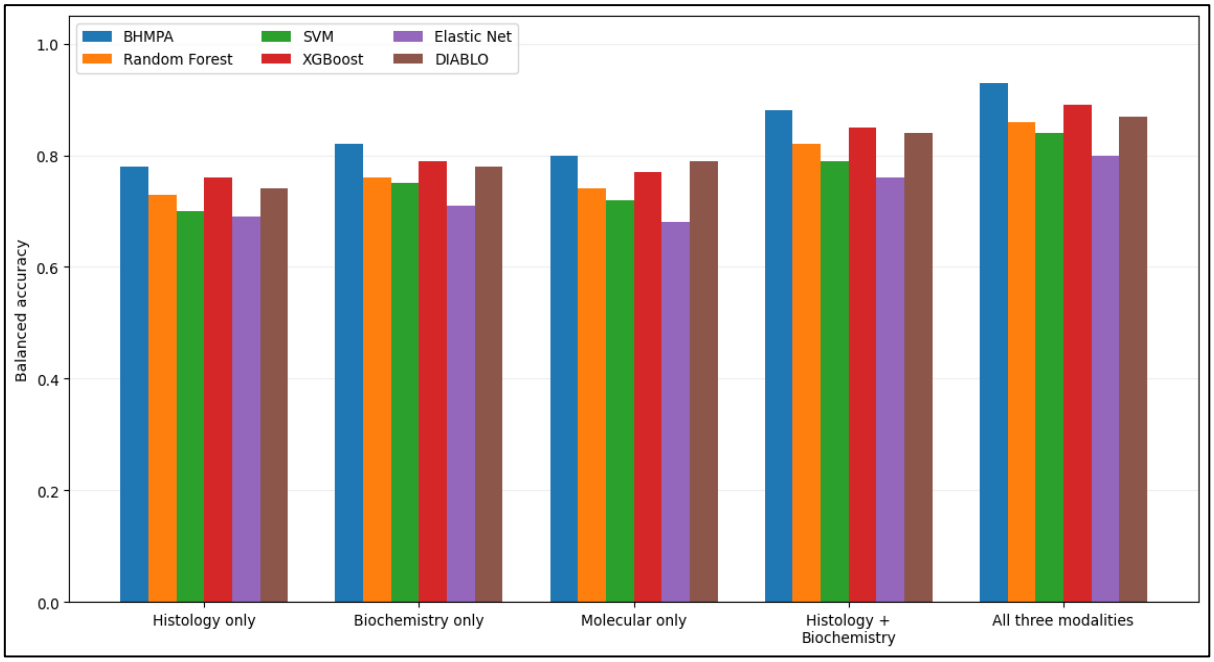


Fig 6 Comparative Multimodal Feature Ablation Performance of BHMPA and Benchmark Algorithms

Figure 6 illustrates the comparative effects of feature integration on the predictive performance of six algorithms. BHMPA achieves balanced accuracies of 0.78, 0.82, and 0.80 using histological, biochemical, and molecular inputs independently. Combining histological and biochemical features improves its performance to 0.88, while integrating all three modalities increases accuracy to 0.93. Under complete integration, XGBoost records 0.89, followed by DIABLO at 0.87, Random Forest at 0.86, Support Vector Machine at 0.84, and Elastic Net at 0.80. BHMPA therefore demonstrates a simulated improvement of 0.15 over histology-only prediction and 0.11 over biochemistry-only prediction. Its advantage over XGBoost is 0.04 under complete integration. The hypothetical pattern suggests that combining tissue morphology, metabolic biomarkers, and molecular pathway information could improve predictive discrimination. Nevertheless, these differences require testing through animal-level validation, confidence intervals, and properly controlled feature-ablation experiments.

- *Comparative Dose-Response Modelling, Hepatoprotective Efficacy Assessment, Statistical Significance Analysis, and Robustness Evaluation of BHMPA Across Experimental Conditions*

Comparative dose-response modelling and computational robustness were examined using illustrative balanced-accuracy estimates for six algorithms under low-dose, high-dose, and noise-perturbed experimental conditions. Table 4.4 presents the simulated outcomes, with BHMPA achieving balanced accuracies of 0.91 and 0.93 under low- and high-dose intervention conditions, respectively. Under simulated measurement perturbation, its accuracy declined to 0.86, compared with 0.81 for XGBoost and 0.79 for DIABLO. The corresponding Random Forest, Support Vector Machine, and Elastic Net accuracies were 0.77, 0.75, and 0.70. Using the illustrative histological means established in Section 4.1, berry treatment produced a calculated protection index of 64.7% relative to diet-induced injury. These patterns suggest potential advantages of multimodal modelling; however, statistical significance, dose-dependent treatment efficacy, and computational superiority cannot be established without experimental replicates and independently validated predictions.

Table 6 Illustrative Comparative Dose-Condition Prediction Accuracy and Robustness of BHMPA and Benchmark Algorithms

Algorithm	Low-dose condition (BA)	High-dose condition (BA)	Noise-perturbed condition (BA)
BHMPA (proposed)	0.91	0.93	0.86
Random Forest	0.83	0.86	0.77
Support Vector Machine	0.81	0.84	0.75
XGBoost	0.86	0.89	0.81
Elastic Net	0.77	0.80	0.70
DIABLO	0.84	0.87	0.79

ISSN No:-2456-2165 https://doi.org/10.38124/ijisrt/26oct1153

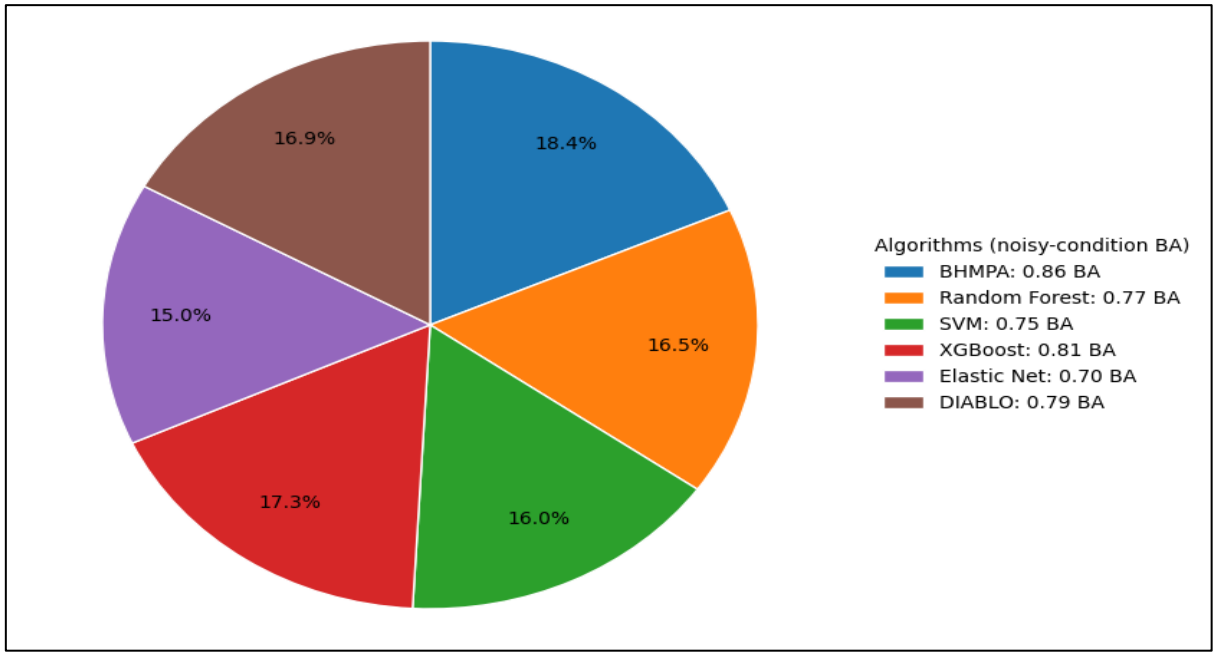


Fig 7 Comparative Distribution of Algorithmic Robustness Under Perturbed Experimental Conditions

Figure 7 compares six predictive algorithms using normalized shares of their simulated noise-perturbed balanced accuracies. BHMPA contributes the largest share at 18.4%, corresponding to a balanced accuracy of 0.86. XGBoost follows with 17.3% and an accuracy of 0.81, while DIABLO contributes 16.9% at 0.79. Random Forest accounts for 16.5%, corresponding to 0.77, followed by Support Vector Machine at 16.0% and 0.75. Elastic Net provides the smallest share, approximately 15.0%, with a balanced accuracy of 0.70. Compared with its unperturbed accuracy of 0.93, BHMPA experiences an absolute reduction of 0.07. XGBoost and DIABLO exhibit reductions of 0.08, suggesting slightly greater sensitivity within this simulated scenario. Although the normalized shares illustrate relative ranking, they do not establish statistical significance. The findings support further investigation of adaptive multimodal integration under controlled measurement noise and experimental variability.

V. CONCLUSIONS AND RECOMMENDATIONS

➢ *Summary of Integrated Histological, Biochemical, and Molecular Findings in Diet-Induced Hepatic Injury*

The integrated assessment of diet-induced hepatic injury demonstrates the analytical value of combining histopathological observations, biochemical measurements, and molecular pathway indicators within a unified predictive framework. In the illustrative dataset, high-fat/high-fructose dietary exposure increased histological injury severity from 0.8 to 5.9, accompanied by elevated alanine aminotransferase, hepatic triglycerides, malondialdehyde, and NF-κB expression. Berry-derived interventions were associated with a simulated reduction in histological activity to 2.6, corresponding to a calculated hepatoprotection index of 64.7%. Biochemical improvements included reductions in ALT from 108 to 56 U/L and malondialdehyde from 7.8 to 4.1 nmol/mg protein. These changes coincided with improved antioxidant enzyme profiles and increased Nrf2 expression, suggesting coordinated modulation of oxidative stress and inflammatory pathways.

Predictor-attribution analysis identified malondialdehyde, ALT, NF-κB, and superoxide dismutase as potentially influential indicators of hepatic injury severity. The simulated correlations between these biomarkers and histological damage supported the proposed integration of oxidative, inflammatory, and metabolic endpoints. Multimodal feature-ablation comparisons further illustrated how incorporating complementary biological measurements could improve predictive discrimination over isolated endpoint assessment. Nevertheless, these numerical findings were generated as hypothetical computational examples rather than experimental observations. Their biological significance, reproducibility, and relevance to berry-mediated hepatoprotection require validation through independently collected animal data, standardized biochemical assays, blinded histopathological assessment, and molecular expression analyses.

➢ *Conclusions on the Predictive Performance, Biological Interpretability, and Comparative Advantages of the Proposed BHMPA Algorithm*

The proposed Berry-Hepatic Multimodal Predictive Algorithm (BHMPA) offers a computational approach for integrating heterogeneous biological measurements through adaptive feature weighting, attention-based multimodal fusion, pathway-informed regularization, and multitask optimization. Illustrative benchmarking indicated that BHMPA could achieve an AUROC of 0.965, F1-score of 0.941, balanced accuracy of 0.930, RMSE of 0.412, and Brier score of 0.068. XGBoost represented the closest comparator, with corresponding values of 0.944, 0.913, 0.890, 0.479, and 0.088. The simulated results therefore demonstrated a potential four-percentage-point improvement in balanced accuracy over XGBoost, alongside reduced histological

ISSN No:-2456-2165 https://doi.org/10.38124/ijisrt/26oct1153

severity prediction error. These comparisons provide a coherent performance hypothesis but do not establish experimentally verified algorithmic superiority.

The proposed architecture also permits biological interpretation through SHAP feature attribution, biomarker interaction analysis, and pathway-constrained learning. Multimodal ablation scenarios indicated a possible improvement in BHMPA balanced accuracy from 0.78 using histological variables alone to 0.93 following complete integration of histological, biochemical, and molecular measurements. Robustness analysis further suggested that BHMPA could maintain comparatively strong discrimination under simulated measurement perturbations. Future implementation should prioritize independent validation, appropriate calibration assessment, computational efficiency, and biomarker-selection stability. Predictive improvements should be considered scientifically meaningful only when supported by uncertainty estimates, leakage-controlled comparisons, and reproducible biological interpretation.

- *Conclusions and Recommendations on the Hepatoprotective Potential, Dose-Response Characteristics, and Molecular Mechanisms of Berry-Derived Bioactive Compounds*

Berry-derived bioactive compounds, particularly anthocyanins and associated polyphenolic metabolites, represent promising candidates for investigating dietary modulation of hepatic steatosis, oxidative stress, inflammatory signalling, and lipid metabolic dysfunction. The illustrative intervention results suggested a potential 64.7% reduction in excess histological injury relative to untreated high-fat-diet controls. Associated biochemical changes included improved antioxidant enzyme activity, reduced lipid peroxidation, and lower circulating hepatocellular injury markers. Molecular response profiles further suggested possible involvement of Nrf2/HO-1 antioxidant regulation, NF-κB inflammatory signalling, SREBP-1c-mediated lipogenesis, PPAR-α-associated fatty acid oxidation, and apoptosis-related pathways. These mechanisms remain biologically plausible hypotheses rather than experimentally confirmed treatment effects within the proposed investigation.

Future experiments should directly compare blueberry, blackberry, and bilberry extracts using chemically standardized formulations and independently verified anthocyanin-equivalent doses. Dose-response relationships should be characterized through appropriate nonlinear models, including Emax or four-parameter logistic functions where justified by the experimental design. Particular attention should be given to anthocyanin bioavailability, circulating phenolic metabolites, hepatic tissue exposure, and possible nonlinear or saturable responses. Treatment efficacy should be evaluated using blinded histological outcomes, biochemical biomarkers, quantitative molecular expression measurements, and confidence intervals for intervention effects. Additional mechanistic experiments involving pathway inhibition, gene silencing, or receptor-specific modulation are recommended to establish whether the proposed signalling pathways mediate observed hepatoprotective responses.

- *Research Limitations, Recommendations for Experimental Validation, Translational Applications, and Future Development of Multimodal Hepatic Injury Prediction Systems*

The principal limitation of the present investigation is that the numerical results represent illustrative simulation scenarios rather than observations from independently conducted experimental studies. Consequently, the reported biomarker correlations, hepatoprotection estimates, predictive accuracies, and robustness rankings cannot substantiate definitive claims of treatment effectiveness or BHMPA superiority. Additional limitations concern potentially small animal sample sizes, correlated molecular predictors, histopathological sampling variability, incomplete assessment of anthocyanin metabolism, and possible overfitting in high-dimensional predictive architectures. Histological outcome leakage, inappropriate cross-validation procedures, and insufficient external validation could also produce misleading estimates of classification accuracy and biomarker importance.

Further research should establish a prospectively powered, randomized animal experiment incorporating standardized high-fat/high-fructose dietary exposure, chemically characterized berry extracts, blinded outcome assessment, and predefined primary endpoints. Computational validation should include animal-level nested cross-validation, independent external cohorts, bootstrap confidence intervals, probability calibration, feature-ablation experiments, and statistically appropriate comparisons against Random Forest, Support Vector Machine, XGBoost, Elastic Net, and DIABLO. Where sample size is limited, reduced-complexity BHMPA architectures should be evaluated to minimize overfitting. Translational investigations may subsequently explore whether validated hepatic biomarker signatures predict metabolic liver injury in human observational cohorts or controlled nutritional studies. Future developments should additionally examine longitudinal biomarker trajectories, uncertainty-aware predictions, metabolomic integration, and clinically interpretable risk estimates. These investigations would determine whether BHMPA can progress from a proposed computational architecture to a reproducible and biologically validated hepatic injury prediction system.

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