ROLE OF PROBIOTICS IN MITIGATING CISPLATIN-INDUCED TOXICITY IN A RAT MODEL

Research project submitted to

Interview Committee for the nomination of

Sun Pharma Science Foundation Science Scholar Awards-2024

Submitted by

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Department of Biotechnology
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Application for Sun Pharma Science Foundation Science Scholar Awards-2024

Evidences and details of all research activities of the work submitted to the Interview Committee for the nomination of

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SUN PHARMA
SCIENCE FOUNDATION

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Benefit for the mankind especially in my case

I would like to share my personal experience At 46 years old, my mother underwent a routine health check-up, which led to an unexpected diagnosis of stage IIIB lung cancer. The situation worsened significantly when it was discovered that the cancer had also spread to her parietal bone, initiating a challenging medical journey involving multiple treatments. This rigorous regimen included four rounds of chemotherapy, a lung lobectomy, and ten sessions of radiation therapy aimed at combating the disease. Initially, she seemed to respond positively to the treatments, bringing a sense of cautious optimism for our family. Unfortunately, this hope was soon dashed when further examinations revealed the cancer's metastasis to her brain, which presented with multiple lesions. This necessitated an additional round of radiation, this time targeting her entire brain, but the extensive progression of the disease at such an advanced stage rendered it incurable. I provided my mother with probiotics supplementation, which appeared to assist her in alleviating pains and mitigating some of the toxic effects associated with cisplatin. The supplementation of probiotics was a proactive measure aimed at enhancing her overall well-being during a particularly trying time. Reflecting on this experience, I recognize the broader mission of supporting individuals afflicted with cancer. Through our family's journey, I have become deeply committed to sharing knowledge and resources that may benefit others who are navigating similar challenges. My aim is to contribute positively to the lives of cancer patients and their families, advocating for treatments and therapies that can alleviate suffering during such a difficult and often heartbreaking ordeal. Despite the extensive treatment efforts, the disease had progressed too far, and this reality was incredibly challenging for both her and our family.

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Contents

List of Figures

List of Tables

Abbreviations

BSA	Bovine serum albumin
CFU	Colony forming units
MRS	De Man, Rogosa and Sharpe bacterial growth medium
OD	Optical density
PBS	Phosphate buffered saline
ROS	Reactive oxygen species
SDS PAGE	Sodium dodecyl sulfate – polyacrylamide gel electrophoresis
WHO	World health organization
DNA	Deoxyribonucleic acid
gDNA	Genomic DNA
mtDNA	Mitochondrial DNA
mRNA	Micro RNA
RNA	Ribonucleic acid
NO	Nitric oxide
AMPs	Antimicrobial peptides
LMWB	Low-molecular-weight bacteriocins
HMWB	High-molecular-weight bacteriocins
AU	Arbitrary unit
mm	Milli meter
LBS	Lactobacillus selection
TS	Tryptic soy broth

BHI	Brain heart infusion broth
GPx	Glutathione peroxidase
GR	Glutathione reductase
SOD	Superoxide dismutase

1. Title

Role of probiotics in mitigating cisplatin-induced toxicity in a rat model

2. Introduction

Cancer is a significant health concern in India, being one of the leading causes of mortality among women aged 30 to 69 years, contributing to approximately 17% of all cancer-related deaths (Zakaria et al., 2024). The World Health Organization (WHO) emphasizes the gravity of the cancer epidemic, particularly within this demographic. One of the major challenges associated with cancer is the late detection of the disease, as around 70% of cases are diagnosed at advanced stages (Laviolette-Brassard 2023). This late diagnosis contributes to high mortality rates and underscores the need for increased awareness and improved diagnostic methods. Cancer treatment varies based on the type and stage of the disease. Traditional approaches include surgery, chemotherapy, and radiation therapy, while newer treatments such as immunotherapy, targeted therapy, hormone therapy, gene therapy have also gained popularity as depicted in Figure. 1. These modern therapies aim to enhance effectiveness and reduce side effects associated with traditional treatments. Among the various treatment modalities, chemotherapy remains a prevalent choice due to its effectiveness in targeting cancer cells. Chemotherapy is an effective and widespread way of cancer treatment in which one more chemotherapeutic or alkylating agents are used. Cisplatin is one of the best and first metal-based chemotherapeutic drugs. Cisplatin is FDA approved for the treatment of advanced cancer; it is a platinum-based chemotherapy drug as shown in Figure. 2 A cell-based high-throughput screening methodology was implemented, using a library of 1,280 Food and Drug Administration (FDA)-approved drugs, to identify clinical compounds that act synergistically with cis-Diamminedichloroplatinum (CDDP).ClS-DIAMMINE DICHLOROPLATINUM (II) (cisplatin), the first heavy metal coordination compound shown to have antineoplastic activity.

Cisplatin, a platinum-based chemotherapeutic drug, is considered one of the foremost metal-based treatments (Lorenzon et al., 2024). It is FDA-approved for advanced cancer treatment and exemplifies the significance of metal-based drugs in oncology.

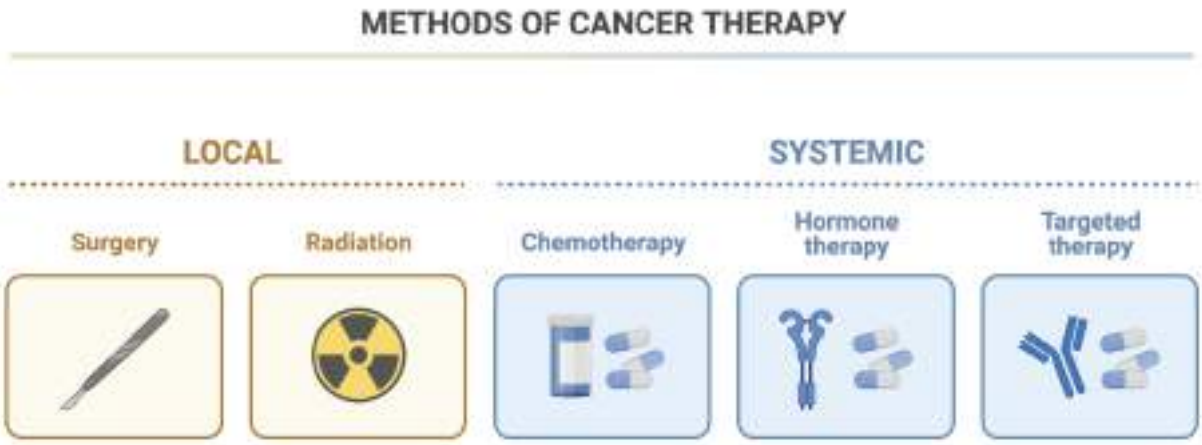

Figure. 1 Different type of treatment options available for cancer

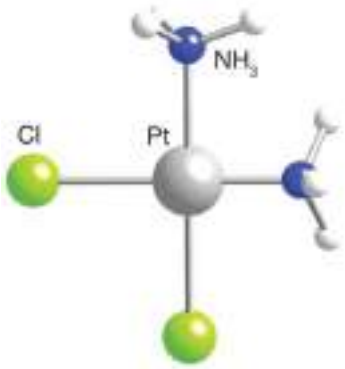

Figure. 2 Structure of Cisplatin - square planar coordination complex

Studies confirmed that cisplatin exerts its anticancer activity by attacking more than one place. It generally binds with genomic DNA (gDNA) or mitochondrial DNA (mtDNA) to create DNA lesions, block the production of DNA, mRNA and proteins, arrest DNA replication, activate several transduction pathways which finally led to necrosis or apoptosis depicted in Figure. 3 (Ghosh, 2019).

However, because of drug resistance and numerous undesirable side effects such as severe kidney problems, allergic reactions, decrease immunity to infections, gastrointestinal disorders, hemorrhage, and hearing loss especially in younger patients it is used in combination therapy (Dasari et al., 2014).

When used in other treatments, chemotherapy can:

- Make a tumor smaller before surgery or radiation therapy.
- Destroy cancer cells that may remain after treatment with surgery or radiation therapy.
- Help other treatments work better. Kill cancer cells that have returned or spread to other parts of your body.

Chemotherapy can cause Side effects - chemotherapy not only kills fast-growing cancer cells, but also kills or slows the growth of healthy cells that grow and divide quickly. Examples are cells that line your mouth and intestines and those that cause your hair to grow. Damage to healthy cells may cause side effects, such as mouth sores, nausea, and hair loss. This research highlights the ongoing efforts to optimize cancer treatment strategies by leveraging with probiotics as therapeutic agents.

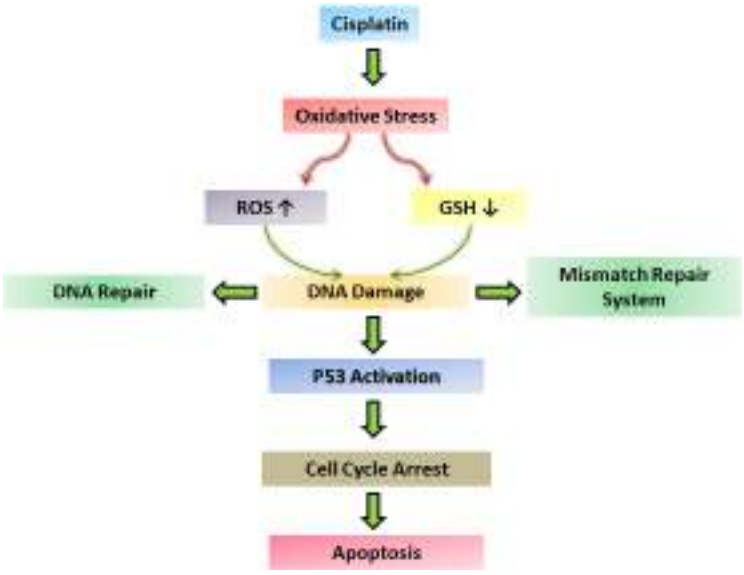

Figure. 3 Overview of molecular mechanisms of cisplatin in cancer treatment.

According to the WHO nutritional guidelines, probiotics can be defined as “live microorganisms when administered in adequate quantities confer a health profit to the host cell” (Nolfo et al., 2013). The use of probiotic applications can stimulate the growth of beneficial microorganisms and out compete potentially harmful bacteria, and thus reinforce the host organism’s natural defense mechanisms (Dimitroglou et al., 2011). Evidence indicates that probiotics can stimulate certain aspects of the innate immune system and may influence oxidative stress, although the mechanisms are only partly described (Tovar-Ramírez et al., 2010). Oxidative stress results from either increased exposure or production of oxygen (reactive oxygen species, (ROS)) and nitrogen (nitric oxide (NO)) radicals, by the organism. Free radicals may induce lipid peroxidation, protein degradation, and DNA damage-inducing apoptotic processes (Sies et al., 1986). The rate or amount of ROS and NO production depends on the metabolic rate of the species under consideration and can be increased by environmental stress (Valavanidis et al., 2006).

Probiotic microorganisms may confer several health benefits to the host as illustrated in Figure. 4. Those effects are the result of three modes of action (Oelschlaeger 2010): i) Immune modulation. Probiotics can exert their modulating effect on the host’s defenses, i.e. both the innate and adaptive immune system. This property might be helpful in the prevention and therapy of infectious diseases and the treatment of inflammatory diseases (Sheil et al., 2007). ii) Microbiota modulation. Probiotics can have a direct influence on other components of the microbiota, either commensals or pathogens. Thus, they can be beneficial in dysbiosis or infectious diseases (Claes et al., 2011). iii) Compounds transformation. Probiotics may modify molecular products produced by other microorganisms, i.e. toxins, produced by the host, i.e. bile salts, or from food origin, i.e. indigestible fibers (Guarner et al., 2017). To exert their beneficial effect on the host, probiotics must remain viable and survive under specific conditions found in the gastrointestinal tract. Different stress tolerance assays can be carried out to assess their ability to survive in those conditions. After being ingested, probiotic bacteria must face stressful conditions in the stomach. In this compartment, pH can vary between 1 and 5, and

several digestive enzymes are secreted. In the same manner, the passage to the duodenum exposes the bacteria to high pH levels, pancreatic enzymes, and bile salts.

Probiotics as a physiological barrier to prevent the attachment of pathogens

Major probiotic mechanisms of action include enhancement of the epithelial barrier, increased adhesion to intestinal mucosa, and concomitant inhibition of pathogen adhesion, competitive exclusion of pathogenic microorganisms, and production of anti-microorganism substances and modulation of the immune system (Bermudez-Brito et al., 2012). The intestinal barrier is a major defense mechanism used to maintain epithelial integrity and to protect the organism from the environment. Probiotics may promote mucus secretion as one mechanism to improve barrier function and exclusion of pathogens.

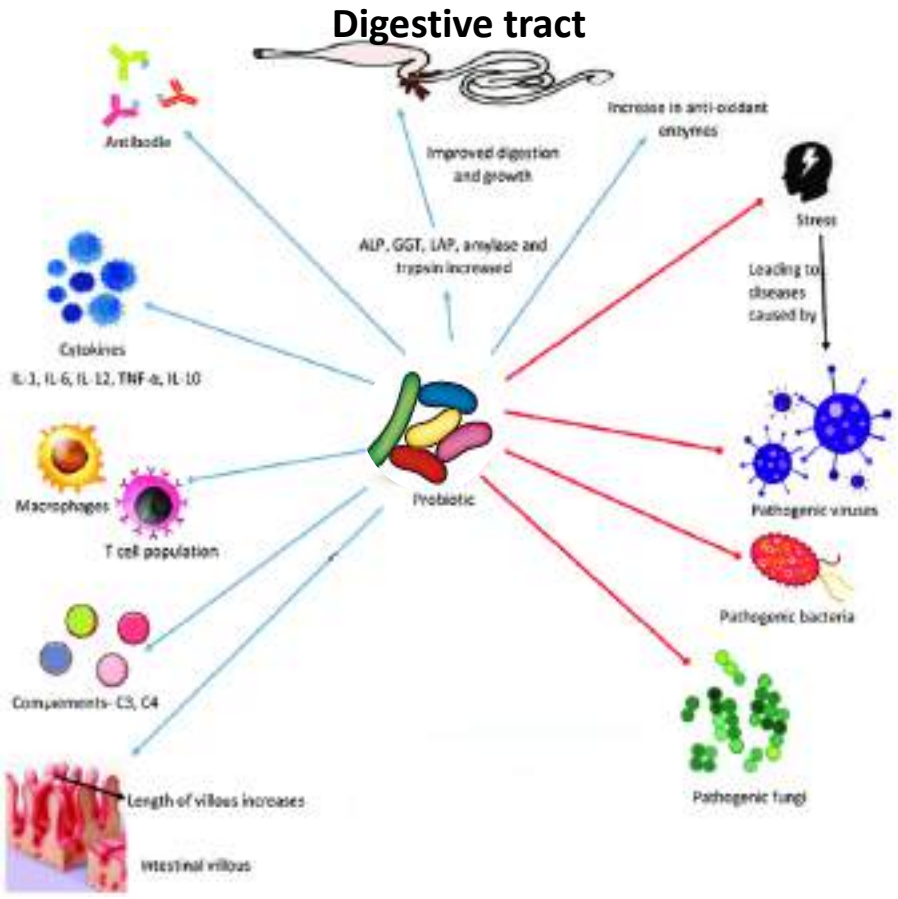

Figure. 4 Beneficial effects of probiotics. Blue arrow indicates additive effects, and red lines indicate inhibitory effect

Impact of probiotics on diarrhea caused by Chemotherapy

In people with cervical cancer, diarrhea is one of the most common and problematic side effects associated with chemotherapy or radiotherapy (Stein et al., 2010; Chitapanarux et al., 2010). The prevalence of these adverse effects was as high as 50-80%. Extreme diarrhea triggered by treatment may contribute to loss of fluids and electrolytes and nutritional deficiencies and may adversely affect the quality of life (Andreyev et al., 2012). In addition, owing to anticancer therapy, patients with neutropenia are more prone to diarrhea, which may also lead to dosage changes or discontinuation or delayed care (Maroun et al., 2007). In preventing or treating chemotherapy-or radiotherapy-induced diarrhea, probiotics can be successful (Qiu et al., 2019). Probiotics have positive effects on the frequency and incidence of diarrhea and the need for rescue treatment in patients undergoing radiotherapy (with or without chemotherapy) (Mego et al., 2013). In the study, patients undergoing radiotherapy with or without chemotherapy were given placebo-related probiotics to avoid diarrhea (Mansouri-Tehrani et al., 2016).

Production of antimicrobial substances

Probiotic strains secrete diverse substances with an inhibitory effect on the growth of pathogenic organisms. The main produced components are lactic acid, hydrogen peroxide, low-molecular-weight bacteriocins (LMWB) and high-molecular-weight bacteriocins (HMWB). Low-molecular-weight bacteriocins are antimicrobial proteins that can be divided in three classes; class I antibiotics, class II heat-stable non-cyclic, and class III cyclic antimicrobial peptides (Maqueda et al., 2008). Bacteriocins are bacterial peptides that inhibit or kill microorganisms. Assays for the evaluation of the inhibitory effect of probiotic-culture filtrates are also performed to assess the effect of secreted bacteriocins and other antimicrobial compounds.

In recent decades, many findings have shed new light on the understanding of the antioxidant capacity of probiotics. Oxidative stress defines a condition in which the prooxidant–antioxidant

balance in the cell is disturbed, resulting in DNA hydroxylation, protein denaturation, lipid peroxidation, and apoptosis, ultimately compromising cells' viability depicted in Figure. 5. ROS-mediated oxidative stress are known to play vital role in the development of chronic diseases such as cancer, diabetes, heart disease, stroke, Alzheimer's disease, rheumatoid arthritis, cataract and aging. Antioxidants are molecules which interact with free radicals generated in cells and terminate the chain reaction before damage is done to the vital molecules (Mishra et al., 2015). Consumption of probiotics alone or in food shows that strain-specific probiotics can present antioxidant activity and reduce damages caused by oxidation (Wang et al., 2017).

Usually, an atom is composed of a central nucleus with a pair of electrons orbiting around it. However, some atoms and molecules have unpaired electrons, and these are called free radicals. Free radicals are usually unstable and highly reactive because of the unpaired electron in its outermost orbit. The unpaired electron makes the radical highly reactive. Free radicals can be stabilized only either by donating electrons to other molecules or by receiving electrons from other molecules. New radicals are generated as a result which can start a chain reaction. Chain reactions can be very destructive. The chain reaction can damage DNA, lipids, proteins. Potential effects of free radicals constitute oxidative stress. Entities causing oxidative stress are called reactive oxygen species (ROS). ROS includes all the free radicals that contain oxygen. The chain reaction stops only when two radicals react and forms a covalent bond. The chain reaction can also be ceased by antioxidants. Antioxidants remove free radicals by getting oxidized themselves. Oxidative stress can damage or kill cells. Oxidative stress may also be responsible for many diseases. Free radicals are produced continuously by the body as a part of a normal physiological process. Function of antioxidant defense system is to keep ROS at an optimum level. Antioxidants include superoxide dismutase, glutathione peroxidase, glutathione reductase, glutathione, catalase, cytochrome c, uric acid etc. Antioxidants act as free radical scavengers and trap free radicals before they can damage tissues.

This study aims to investigate the protective effects of probiotics on cisplatin-induced toxicity in rats, focusing on hematological, biochemical, and histopathological parameters. The importance of

this research lies in its potential to provide a novel adjunct therapy to conventional chemotherapy, leveraging the beneficial properties of probiotics to mitigate cisplatin's adverse effects. The findings will contribute to a deeper understanding of the mechanisms through which probiotics exert their protective effects, emphasizing their role in maintaining physiological and biochemical homeostasis during chemotherapeutic stress. This study not only underscores the therapeutic potential of probiotics in oncology but also paves the way for future clinical trials and applications in cancer treatment protocols.

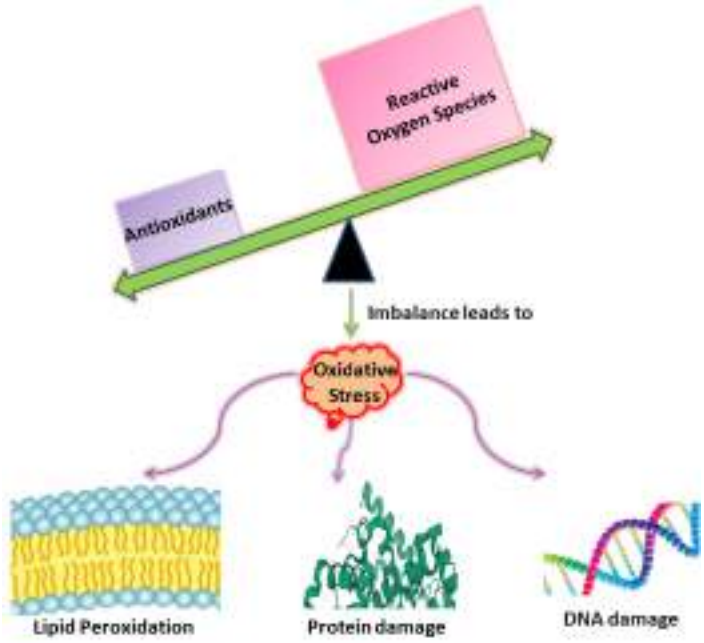

Figure. 5 Antioxidants are required for balancing ROS which cause oxidative stress.

3. Aim and Objectives

The present study was planned to isolate and characterize the bacteriocin from probiotic and to investigate the protective mechanism of probiotics against cisplatin induced toxicity in Albino rats. Therefore, the present study included:

1. To isolate and characterize probiotic strains from raw goat milk.

2. To investigate the haematological indices after treatment with cisplatin and probiotics (individual and combined effects)
3. To evaluate carbohydrate, lipid, and protein metabolism in albino rats exposed to different treatments
4. To assess redox enzyme activities and antioxidative enzymes in the experimental groups
5. To analyze oxidative stress and DNA damage in albino rats.

4. Materials and methods

Sample collection

Goat milk samples were collected from different goat farms in Tirupati. The samples were aseptically transferred to sterilized falcon tubes and then transported to the lab.

Isolation of bacterial strains

The milk sample is then serially diluted and spread onto selective agar plates such as MRS (de Man, Rogosa, and Sharpe) agar medium. To get pure cultures, selected isolates were cultured using the streak plate technique and incubated anaerobically at 37ºC for 24-48 hrs. The selected isolates were sub-cultured in MRS agar slants based on their performance in MRS media. These slants were incubated for 24 hours at 37°C and stored at −20°C in 20% glycerol before further use. This study aims to isolate probiotics from sources of natural origin that are easily accessible in the area, despite the existence of other dairy products such as cheese, milk yogurt, and processed milk. Consequently, probiotic isolation did not include commercial products that were on the market.

Morphological characterization

Using standard microbiology techniques, the isolated colonies were evaluated for their morphological properties and biochemical tests. Pure isolates were identified based on their macroscopic appearance, including the size, shape, margin, color, and texture of colonies. Gram staining of bacterial colonies was performed using a Hi-Media kit and was examined under a light microscope with 100X

magnification. Gram-positive bacterial colonies were tested for KOH, catalase, oxidase, IMVIC (Indole, Methyl Red, Voges Prausker, and Citrate Utilization), arginine hydrolysis, nitrate reduction, acid tolerance, bile slats, adhesion, anti-bacterial assay, and aggregation assays.

Molecular characterization by 16s rRNA sequencing

The isolated strains were then subjected to molecular and taxonomy study. The consensus sequence obtained was used for species identification by BLAST (Basic local alignment search tool) in NCBI-GENBANK. The sequence has been added to the NCBI database.

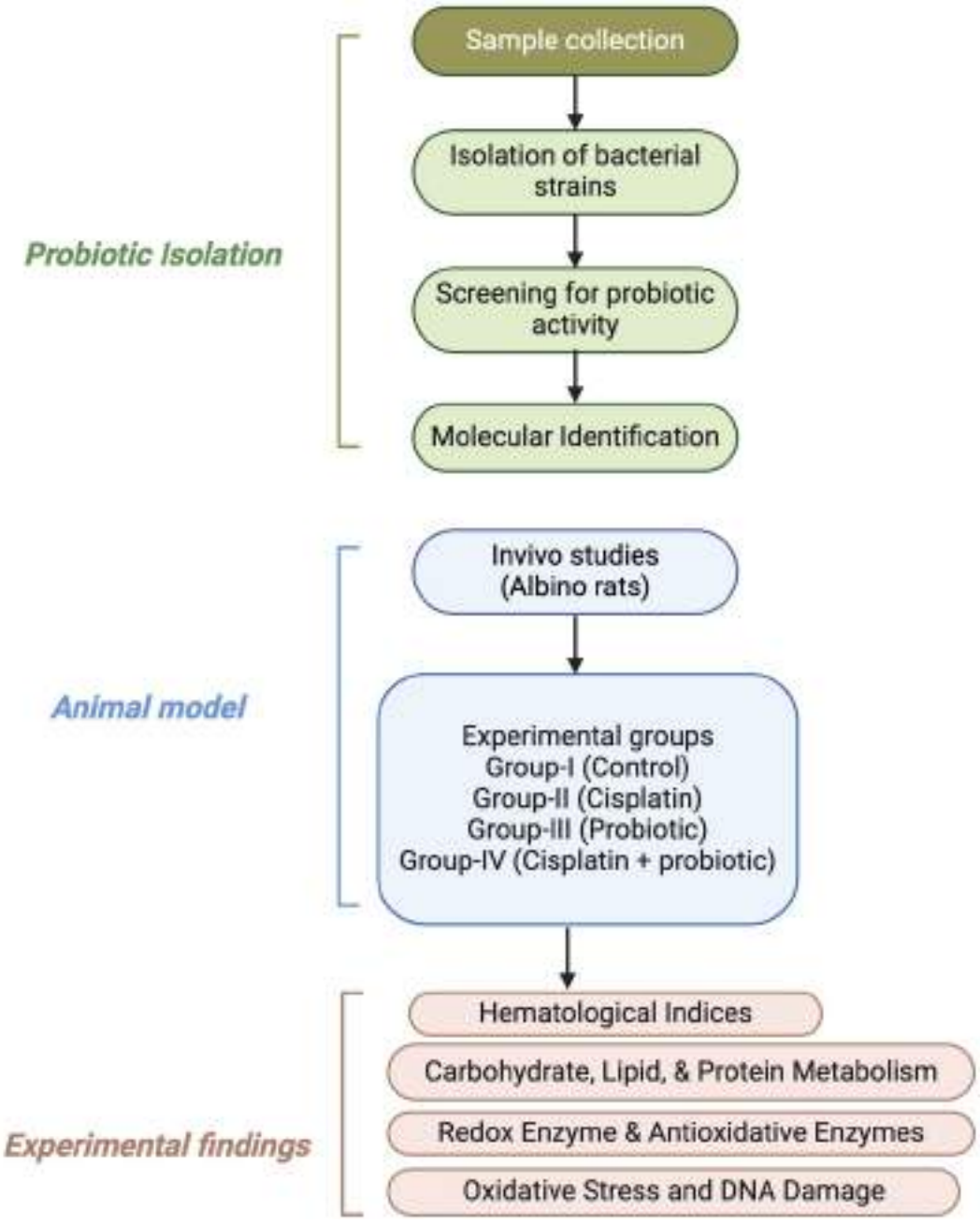

Figure. 6 Overview of the work done

Experimental animals

All albino rats were handled in compliance with the Institutional Ethical Committee's guidelines for the care and use of laboratory animals of Sri Padmavati Mahila Visvavidyalayam (Proposal No. CPCSEA/1677/SPMVV/IAEC/III-07, dated 10-03-2023), India. Healthy female albino rats, 24 in number, weighing between 150–200 g, were procured from Sri Venkateswara Enterprises, Bangalore, for this study. They were housed in polycarbonate cages under standard laboratory conditions at a room temperature of 24 ± 2°C and humidity of 45-64% with a 12-hour light/dark cycle. The rats were fed a standard diet obtained from Sri Venkateshwara Enterprises, Bangalore, India, and water was supplied through plastic bottles equipped with nipples.

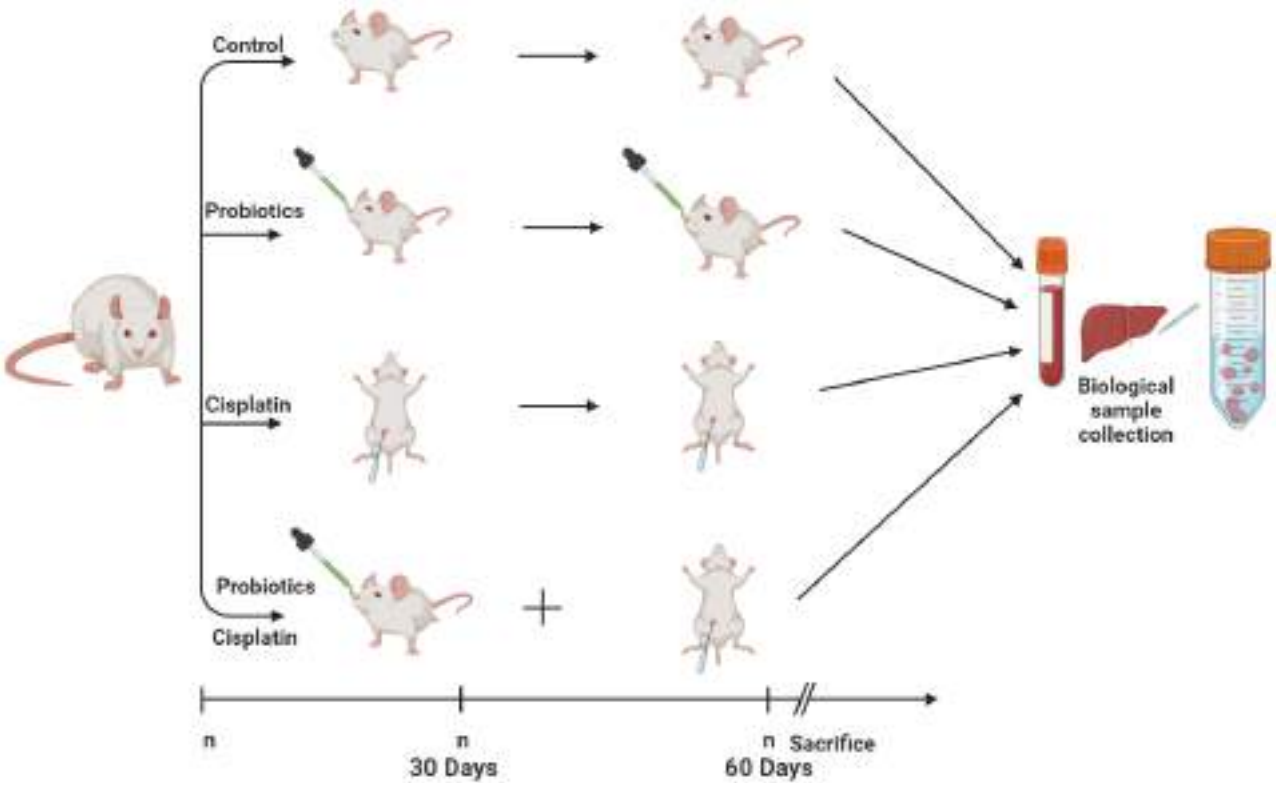

Figure. 7 Experimental Setup for Evaluating the Protective Effects of Probiotics in a Cisplatin-Induced Ovarian Toxicity Rat Model

Group I (G1) – served as a control, fed solely a commercial diet.

Group II (G2) - Cisplatin (5mg/kg) injected by intraperitoneal (IP) injection in total, six times—once every week—during the 45-day course of treatment. The following concentrations of cisplatin were selected for LC_{50} determination: 1.0, 1.5, 2.0, 2.5, 3.0, 3.5, 4.0, 4.5 and 5.0 mg/kg.

Group III (G3) – Treated with probiotics (*B. subtilis)* via oral gavage at a concentration of 10^9 CFU).

Group IV (G4) - Probiotic + cisplatin, probiotic was given orally, and cisplatin was given by IP injection along with the treatment.

Table. 1 Outlines the experimental design used to assess the protective effects of probiotics against cisplatin-induced ovarian toxicity in rats

No. of groups	Groups	Animals	R. O. A	Dose	Treatment days
Group 1	**Control**	**6**	**-**	**-**	**-**
Group II	**Cisplatin**	**6**	**IP**	**5 mg/ kg**	**45 days**
Group III	**Probiotic**	**6**	**Oral**	**10^9 CFU/mL**	**45 days**
Group IV	**Probiotic + Cisplatin**	**6**	**Oral+IP**	**10^9 CFU/mL +5 mg/ kg**	**45 days**

After exposure, blood samples were collected from the animals and euthanized. The liver, kidney, and ovarian tissues were immediately collected and stored in 10% formalin to retain their integrity and avoid degradation as represented in Figure. 7. These preserved tissues were subsequently used for further biochemical experiments to ensure reliable test findings.

Evaluation of Hematological Parameters

This study evaluates the hematological parameters of control and treated rats to understand the physiological impacts of the treatment. Differential white blood cell counts, Platelet and White blood cell analysis, and red blood cell analysis were performed. Using an automated hematological analyzer, the values were determined.

Estimation of carbohydrate metabolism

Carbohydrate metabolism in tissue homogenates is assessed by measuring the levels of major

enzymes involved in carbohydrate metabolic pathways such as glycolysis, gluconeogenesis, the citric acid cycle, and the pentose phosphate pathway. The estimation of the total carbohydrate content was made using the Carrol et al., (1956) approach in the liver, kidney, and ovarian tissues of female albino rats treated. The lactic acid levels were measured by Barker et al., (1941), later modified by Huckabee et al., (1961). Using Friedemann et al., (1942) method the pyruvic acid content in homogenates was estimated. The total amount of glucose in the liver, kidney, kidney, and ovarian tissues of both control and treated albino rats was calculated using Kemp and Van Heijningen's (1954) method.

Estimation of Lipid profile

The lipid profile was estimated through standard enzymatic assay kits. The total cholesterol in the serum was determined using a cholesterol quantification kit (MAK043-Sigma). Likewise, high-density lipoproteins (HDL) and low-density lipoproteins (LDL) were quantified using HDL and LDL Quantitation Kit (MAK045-Sigma). Similarly, a Triglyceride Quantification Colorimetric/Fluorometric Kit (MAK266-sigma) was used to measure the triglyceride amount.

Estimation of protein content

Lowry et al., (1951) method was used to quantify the total proteins, structural proteins and soluble proteins in liver, kidney, and ovarian tissues using bovine serum albumin (BSA) as standard. Using the method described by Schurr et al., (1950), free amino acids from the homogenate were extracted and measured. Standard curves for extensive routine amino acid investigations were created from a composite reference. Protein content was expressed as mg/g wet weight.

Estimation of redox enzyme activities

Measuring redox enzyme activities includes preparing homogenates of 10% w/v liver, kidney, and ovarian tissues in an ice-cold 0.25 M sucrose solution, the samples were centrifuged at 10,000 rpm for 15 minutes at 4°C. The resultant supernatant has been utilized to perform enzymatic tests for lactate dehydrogenase (LDH), succinate dehydrogenase (SDH), and glucose-6-phosphate dehydrogenase (G-6-PDH). LDH activity was assessed by a spectrophotometer at 495 nm by measuring formazone production, using an improved approach from Nachlas et al. (1960) as described by Pramilamma et al., 1975. SDH activity was also measured at 495 nm using Nachlas et

al.'s (1960) method, which used flavin adenine dinucleotide (FAD) and Iodonitrotetrazolium chloride (INT) to create formazone. G-6-PDH activity was determined using a technique adopted by Lohr and Waller (1965) and refined by Mastanaiah et al. (1978). All enzyme activities were represented in µ moles of formazone formed µmol/min/mg. By catalysis conversion of oxidized glutathione (GSSG) to reduced glutathione (GSH) for the consumption of NADPH, the catalytic activity of glutathione reductase was measured spectrophotometrically at 340 nm. The specific activities were expressed as the nmol of NADPH consumption per min per mg of protein (Carlberg and Mannervik, 1975)

Assessment of antioxidant assays

Glutathione peroxidase (GPx) activity was estimated using the method of Carlberg and Mannervik (1975). The unit of GPx activity was expressed as micromoles of NADPH oxidized per minute. It catalyzes the reduction of hydrogen peroxide (H_2O_2) and organic hydroperoxides to water (H_2O) and corresponding alcohol, which was measured at 340 nm using a spectrophotometer. The activity of superoxide dismutase was determined in the tissue homogenates by the modified method of NADH-phenazine methosulphate-nitroblue tetrazolium formazan inhibition reaction spectrophotometrically, measured at 550 nm (Kakkar et al. 1984). The catalytic activity of catalase was determined by a spectrophotometrical measurement of H_2O_2 breakdown at 240 nm. The specific activity of the enzyme was expressed as μmol of decomposed H_2O_2 per min per mg of protein (Aebi, 1984).

Estimation of oxidative stress

ROS scavenging activity

Intracellular ROS scavenging activity was determined by taking around 2 mg of tissue and mashed in PBS buffer. Subsequently, 1 mL of the tissue solution was mixed with 250 µM of quinol and allowed to incubate for 30 to 45 minutes, the UV-vis spectrophotometer was used to determine the concentration at 305 nm. Using hydrogen peroxide (H_2O_2) as a standard as outlined in the Ruch et al., 1989 method, the proportion of ROS activity was first assessed. The percentage of ROS activity was estimated using the following formula:

$$\text{The ROS scavenging activity percentage} = \frac{A_C - A_T}{A_C} X\ 100 \quad \ldots\ldots\ldots\ldots\ (1)$$

where A_C = Absorbance of control, A_T = Absorbance of sample.

Lipid peroxidation: An end product of lipid peroxidation is MDA (Malondialdehyde), which was measured in tissue homogenates based on the reaction with thiobarbituric acid (TBA) to form a pink color complex, MDA produced was determined with the absorbance coefficient of the MDA-TBA complex at 550 nm using 1, 1, 3,3-tetraethoxypropane (TMP) as the standard (Okhawa et al. 1979).

Determination of DNA damage by 8-OHdG and Caspase-3 Levels

DNA damage was assessed using an 8-Hydroxydeoxyguanosine (8-OHdG) ELISA kit by Thermo Fisher Scientific following the manufacturer's instructions. Caspase-3 levels in liver, kidney, and ovarian tissues were evaluated using the EnzChek Caspase-3 Activity Assay Kit from Thermo Fisher Scientific by the guidelines provided in the kit.

Histopathology

The liver, kidney, and ovary tissues were preserved in 10% formalin for standard histopathological assessments. The tissues were subsequently fixed for hematoxylin and eosin (H&E) staining using the technique outlined by Bano and Najam (2019).

Statistical analysis

All experimental data reported in the results are the means of triplicate measurements. The collected findings were evaluated and compared using Duncan's new Multiple range (DMR) test and reported differences were statistically significant at $P < 0.05$.

5. Results

Screening and isolation of bacterial strains

Initially, 6 bacterial colonies were isolated from goat milk samples, predominantly identified by morphological and biochemical assays (Table. 2). The bacterial isolates were gram-positive, bile salts, NaCl tolerance, acid tolerance, and antibacterial assay positives. Then, one isolate named C3 was chosen for molecular identification out of the 6 isolates. The 16S rRNA gene sequence analysis of this isolate disclosed that this bacterium belongs to *B. subtilis* and the isolate's 16S rRNA gene sequence was submitted to the NCBI database (National Center for Biotechnology Information) with

the accession number PP472092.

Table. 2 Biochemical characterizations of isolates from goat milk. Plus (+) and minus sign (−) indicate the positive and negative results of reaction/test, respectively.

Basic Characteristics	C-3	C5	C2	C2.1	C9	C6
Catalase	-	-	+	+	-	+
Citrate	+	-	+	-	+	+
Gelatin Hydrolysis	-	+	+	-	+	-
Gram Staining	+	+	+	+	+	+
Indole	-	+	-	+	+	-
MR (Methyl Red)	-	+	+	-	+	-
Voges-Proskauer	-	+	+	-	-	-
Bile salts	+	-	-	+	+	+
NaCl tolerance	+	-	-	+	+	+
Acid tolerance	+	-	-	+	+	+
Antibacterial activity	+	-	-	-	-	+

Experimental group outcomes

Estimation of haematological parameters

The effects of probiotics and cisplatin on different blood parameters were investigated in this study. All treatment groups exhibited an increase in lymphocytes compared to the control in the white blood

cell differential counts cisplatin obtained the highest percentage (82.52 ± 4.12) within the normal range of 60-86%. Monocytes in the cisplatin group showed a slight rise of 0.85 ± 0.01 while other groups are within the usual 0-1% range. While basophil levels were steady in all groups, eosinophil counts were slightly increased in the cisplatin group (2.43 ± 0.15) as mentioned in Table.1. Serum biochemical analytes, revealed that the cisplatin group noted an enormous increase in glucose levels (123.35 ± 5.45), exceeding the usual range. Likewise in the cisplatin group, total cholesterol (72.23 ± 4.41), both HDL, LDL, and BUN levels (35.23 ± 2.12) showed a significant increase. While the probiotic (6.4 ± 0.21 × 10^6/µL) and combination groups (6.5 ± 0.23 × 10^6/µL) stayed within the normal range, the RBC count was lower in the cisplatin group (5.37 ± 0.48 × 10^6/µL) than in the control group (6.1 ± 0.32 × 10^6/µL). In comparison to the control group (13.3 ± 0.81 g/dL), probiotic group (15.3 ± 0.92 g/dL), and combination group (14.12 ± 1.45 g/dL), haemoglobin levels fell dramatically in the cisplatin group (7.15 ± 0.42 g/dL). PCV was within the normal range in the cisplatin group (15.3 ± 1.5%), probiotic group (31.85 ± 1.27%), control group (27.21 ± 1.54%), and combination group (28.6 ± 3.26%). The cisplatin group had a higher PLT count (2.52 ± 0.03 × 10^5/µL) than the probiotic (1.16 ± 0.04 × 10^5/µL), combination (1.38 ± 0.03 × 10^5/µL), and control groups (1.23 ± 0.05 × 10^5/µL). Similarly, the WBC count increased considerably in the cisplatin group (12500 ± 45.34 per mm^3) as compared to the control (6700 ± 34.74 per mm^3), probiotic (7280 ± 35.66 per mm^3), and combination groups (9120 ± 16.23 per mm^3). Overall, the administration of cisplatin resulted in significant impacts on RBC and PCV and rises in glucose, cholesterol, HDL, LDL, BUN, and WBC levels. When probiotics and cisplatin were administered together, the outcomes were controlled, which may imply that probiotics may reduce the effects of cisplatin-induced alterations.

Estimation of carbohydrate metabolism

The control group's total carbohydrates were 72.54 mg/g in the kidneys, 52.54 mg/g in the liver, and 78.64 mg/g in the ovaries. These levels were dramatically lowered to 48.32 mg/g, 41.62 mg/g, and 28.32 mg/g, respectively, after receiving cisplatin treatment as shown in Figure. 8. The probiotic treatment-maintained levels close to the control, with levels of 79.97 mg/g in ovaries, 75.97 mg/g in

kidneys, and 52.97 mg/g in liver. Cisplatin and probiotics together exhibited intermediary levels ranging from 72.44 mg/g, 70.14 mg/g, and 50.44 mg/g. Lactic acid levels increased to 58.25 mg/g, 39.25 mg/g, and 34.25 mg/g, respectively, after cisplatin treatment when compared to the probiotic group (31.14 mg/g, 29.14 mg/g, and 25.14 mg/g). In comparison to cisplatin alone, the combined treatment demonstrated lower levels in the liver, kidneys, and ovaries, with 32.41 mg/g, 27.41 mg/g, and 28.41 mg/g. In terms of pyruvic acid, the levels had been substantially reduced in cisplatin ovaries, kidneys, and liver (12.12 mg/g, 11.12 mg/g, and 9.12 mg/g). The probiotic treatment exhibited (32.74%, 31.97%, and 30.47 mg/g) that were slightly higher than the control. Probiotics and cisplatin together resulted in levels of 25.14 mg/g in the liver, 30.49 mg/g in the kidneys and 29.15 mg/g in the ovaries. Moreover, the cisplatin group lowered glucose levels (5.91, 6.17, and 5.11) compared to control ovaries, kidneys, and liver. However, probiotics group was almost in the range of the control group (10.89, 9.79, and 7.19). The results of combination treatments were in a moderate range (9.67, 8.99, and 6.99) in all three groups respectively.

Estimation of Lipid profile

The effects of probiotic supplements and cisplatin treatment on the lipid profiles of the ovaries, kidneys, and liver. Total cholesterol levels varied depending on the treatment: probiotic intake showed 55.6 mg/dL, 66.84 mg/dL, and 80.98 mg/dL; combined treatment resulted in 52.13 mg/dL, 60.12 mg/dL, and 78.57 mg/dL. Control group exhibited 58.67 mg/dL (ovaries), 63.2 mg/dL (kidneys), and 81.4 mg/dL (liver) these were increased to 79.5 mg/dL, 90.56 mg/dL, and 108.47 mg/dL, respectively in the cisplatin-treated group. Similar trends were seen in high-density lipoproteins (HDL) control group exhibited 41.23 mg/dL for the ovaries, 49.56 mg/dL for the kidneys, and 55.46 mg/dL for the liver; cisplatin decreased to 24.69 mg/dL, 21.67 mg/dL, and 32.87 mg/dL when compared to probiotic treatment group 43.54 mg/dL, 51.22 mg/dL, and 57.73 mg/dL and combined group showed 40.13 mg/dL, 45.87 mg/dL, and 51.46 mg/dL. Triglycerides (TG) and low-density lipoproteins (LDL) in all organs showed comparable patterns during therapy as depicted in Figure. 9, indicating different effects of probiotic and cisplatin therapies on lipid metabolism.

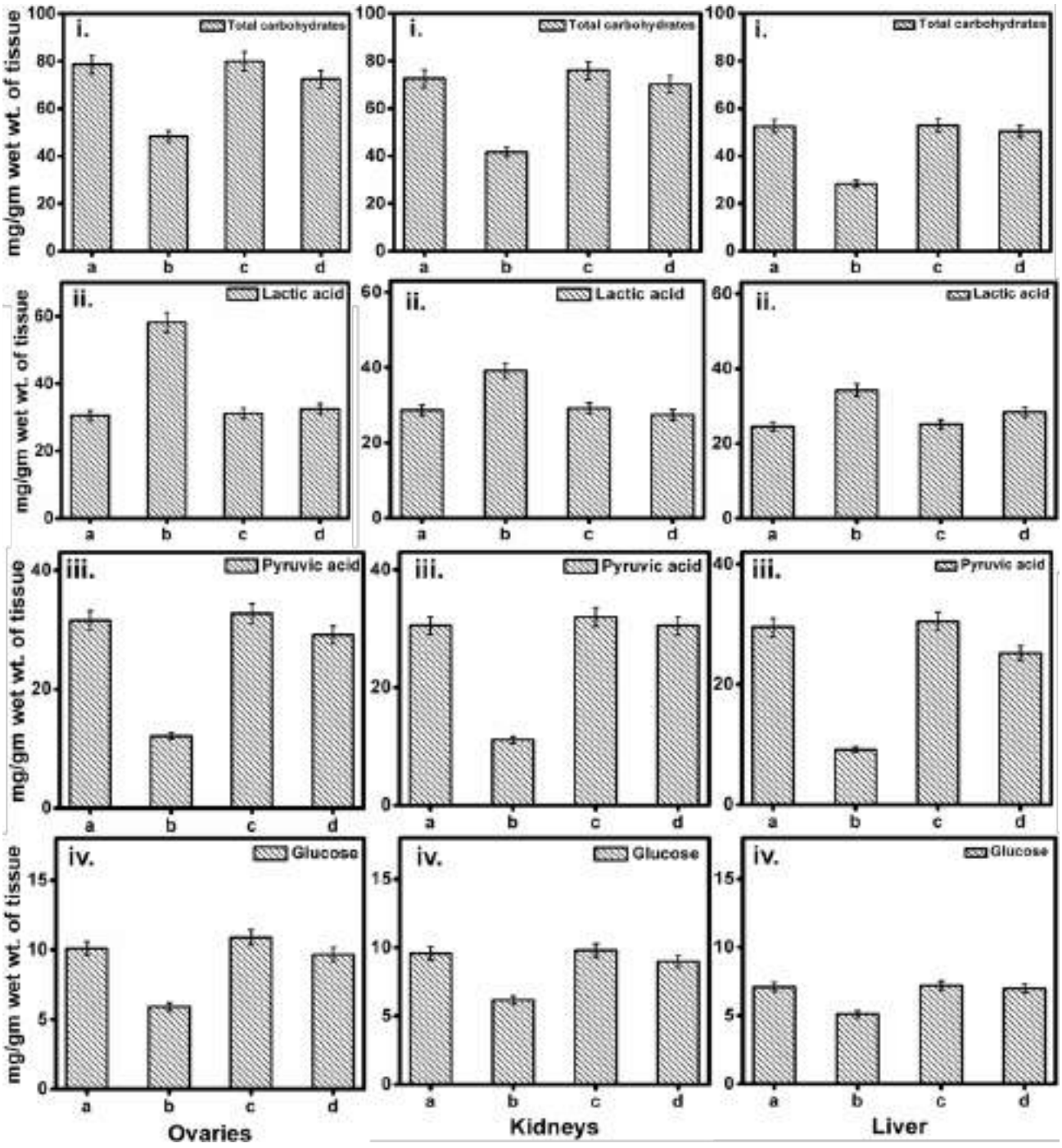

Figure. 8 Levels of carbohydrates metabolism i. Total carbohydrates ii. Lactic acid iii. Pyruvic acid iv. Glucose in Ovaries, Kidneys, and Liver after treatment.

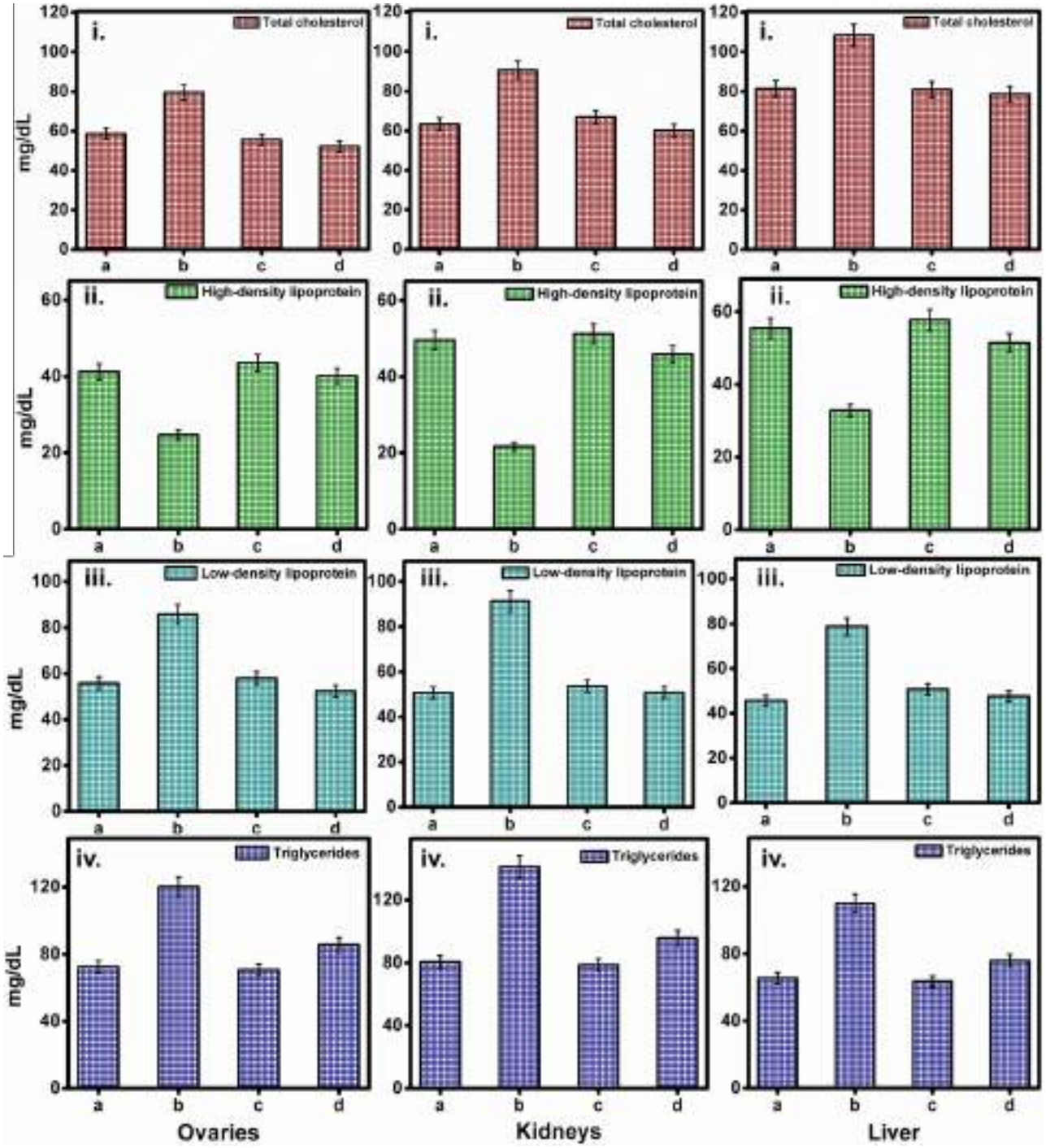

Figure. 9 Levels of Lipid profile i. Total cholesterol ii. High-density lipoprotein iii. Low-density lipoprotein iv. Triglycerides in Ovaries, Kidneys, and Liver after treatment.

Estimation of protein content

The control group exhibited total protein levels in the kidneys (72.54 mg/g), liver (164.45 mg/g), and ovaries (174.67 mg/g), while the cisplatin treatment group displayed lower levels in the kidneys (41.62 mg/g), liver (140.05 mg/g), and ovaries (142.15 mg/g). In comparison to controls, probiotic treatment typically maintained or slightly raised total protein levels, with the liver 165.01 mg/g,

kidneys 75.97, and ovaries 175.53 mg/g. Total protein levels in the ovaries (168.82 mg/g), kidneys (70.14 mg/g), and liver (163.00 mg/g) showed varying levels when cisplatin and probiotics were combined. The same trends were seen in structural proteins in the liver (79.12 mg/g), kidneys (38.64 mg/g), and ovaries (79.14 mg/g), cisplatin therapy decreased protein levels in the liver (71.99 mg/g) and kidneys (19.25 mg/g) but not in the ovaries (61.56). Probiotic supplementation in the liver (81.09 mg/g), kidneys (39.14 mg/g), and ovaries (79.64 mg/g) raised structural protein levels. The effects of cisplatin when combined with probiotics were mild for the liver (77.6 mg/g), kidneys (37.41 mg/g), and ovaries (74.52 mg/g). Similar patterns were observed for soluble proteins control group exhibited in the kidneys (23.52 mg/g), liver (85.74 mg/g), and ovaries (86.97 mg/g); cisplatin-treated group decreased levels in the kidneys (11.12 mg/g), liver (76.54 mg/g), and ovaries (72.67 mg/g); probiotic group increased in the kidneys (21.97 mg/g), liver (86.21 mg/g), and ovaries (86.54 mg/g). In the combination group soluble proteins in kidneys (19.49 mg/g), ovaries (84.23 mg/g), and liver (86.01 mg/g). There was an increase in free amino acid levels in the cisplatin group's kidneys (8.17 mg/g), liver (9.45 mg/g), and ovaries (7.27 mg/g) compared to the control group (4.98 mg/g, 4.58 mg/g, and 6.25 mg/g) as outlined in Figure. 10.

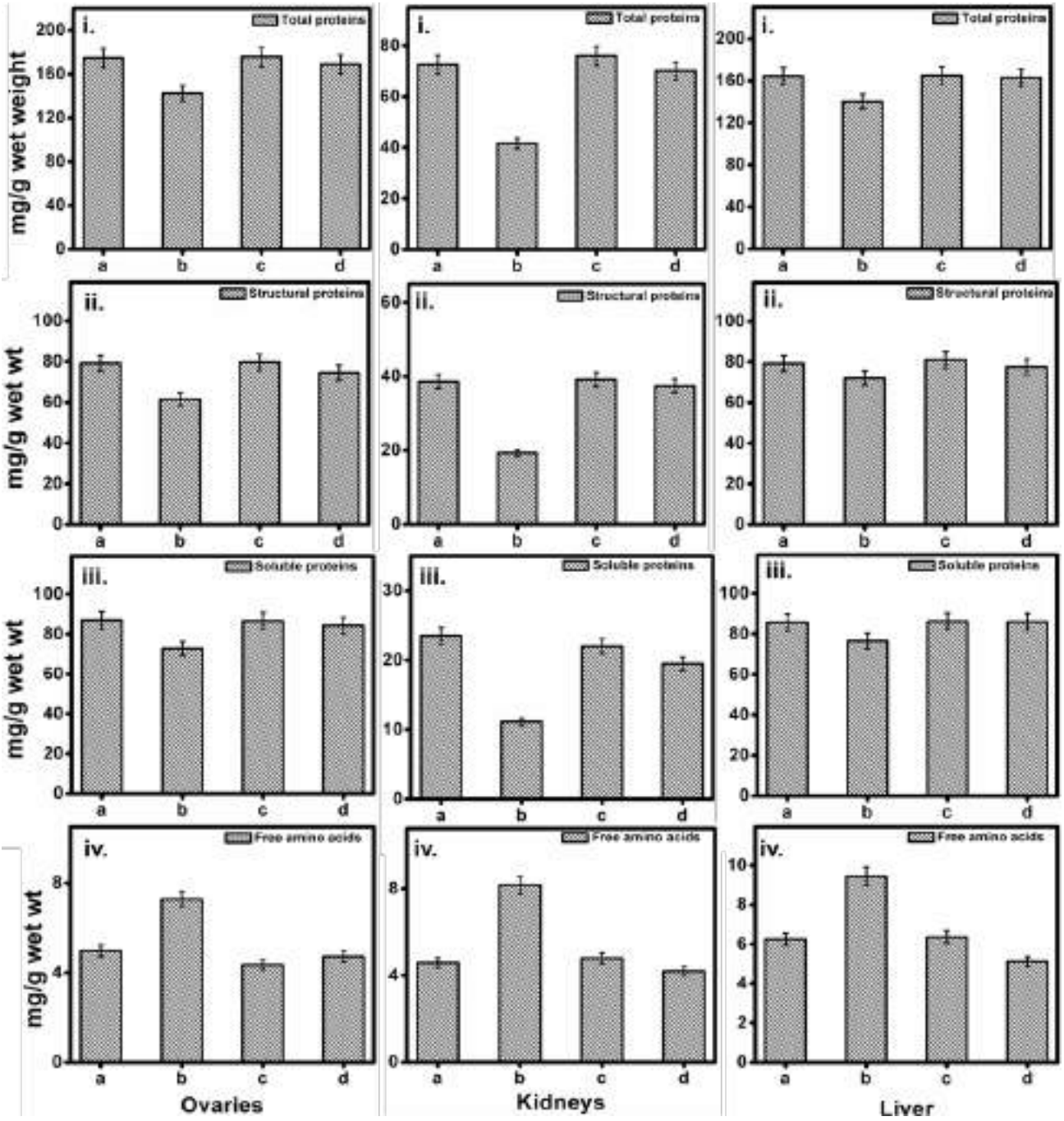

Figure. 10 Levels of protein metabolism i. Total proteins ii. Structural proteins iii. Soluble proteins iv. Free amino acids in Ovaries, Kidneys, and Liver after treatment.

Estimation of redox enzyme activities

The activities of SDH, LDH, G-6-PDH, and GR were assessed under control, cisplatin, probiotic, and cisplatin and probiotic conditions. The control group's liver, kidneys, and ovaries displayed SDH activity levels of 10.96, 8.64, and 8.21 µmol/min/mg, respectively. The cisplatin group exhibited considerably lower activity levels of 2.74, 2.54, and 2.85 µmol/min/mg. The probiotic treatment

produced levels of 8.94, 8.73, and 11.16 which were marginally higher than the control. The probiotic + cisplatin group exhibited in between levels of activity of 7.56, 8.00, and 9.85 µmol/min/mg. The control group's LDH activity levels in the liver, kidneys, and ovaries were 2.51, 6.24, and 6.23 µmol/min/mg. After receiving cisplatin, these levels decreased to 1.53, 2.56, and 1.54 µmol/min/mg. The activities were increased by the probiotic therapy to 2.76, 6.39, and 6.31 µmol/min/mg, whereas the levels in the cisplatin plus probiotic group were 2.18, 5.87, and 5.96 µmol/min/mg. In the ovaries, kidneys, and liver, the control group showed G-6-PDH levels of activity of 3.62,4.34, and 4.38 µmol/min/mg respectively. In response to cisplatin treatment, these levels decreased to 1.62, 1.41, and 1.91 µmol/min/mg. The probiotic group showed marginally increased activity of 3.82, 4.56, and 4.69 µmol/min/mg. Cisplatin combined with probiotics yielded levels of 3.29, 4.00, and 4.12 µmol/min/mg as illustrated in Figure. 11. Lastly, GR activity in the kidneys, liver, and ovaries was 0.72, 1.62, and 0.84 nmol min^{-1} mg $protein^{-1}$ in the control group. Following cisplatin treatment decreased to 0.44, 0.35, and 0.55 nmol min^{-1} mg $protein^{-1}$. The activities were somewhat elevated to 0.85, 0.86, and 1.68 nmol min^{-1} mg $protein^{-1}$ in the probiotic group. The activities in the group receiving cisplatin with probiotics were 0.828, 0.94, and 1.59 nmol min^{-1} mg $protein^{-1}$. The results of the study show that probiotics, especially when administered in combination with cisplatin, serve to maintain or slightly increase enzyme activities, while cisplatin drastically inhibits enzyme activities across all tissues.

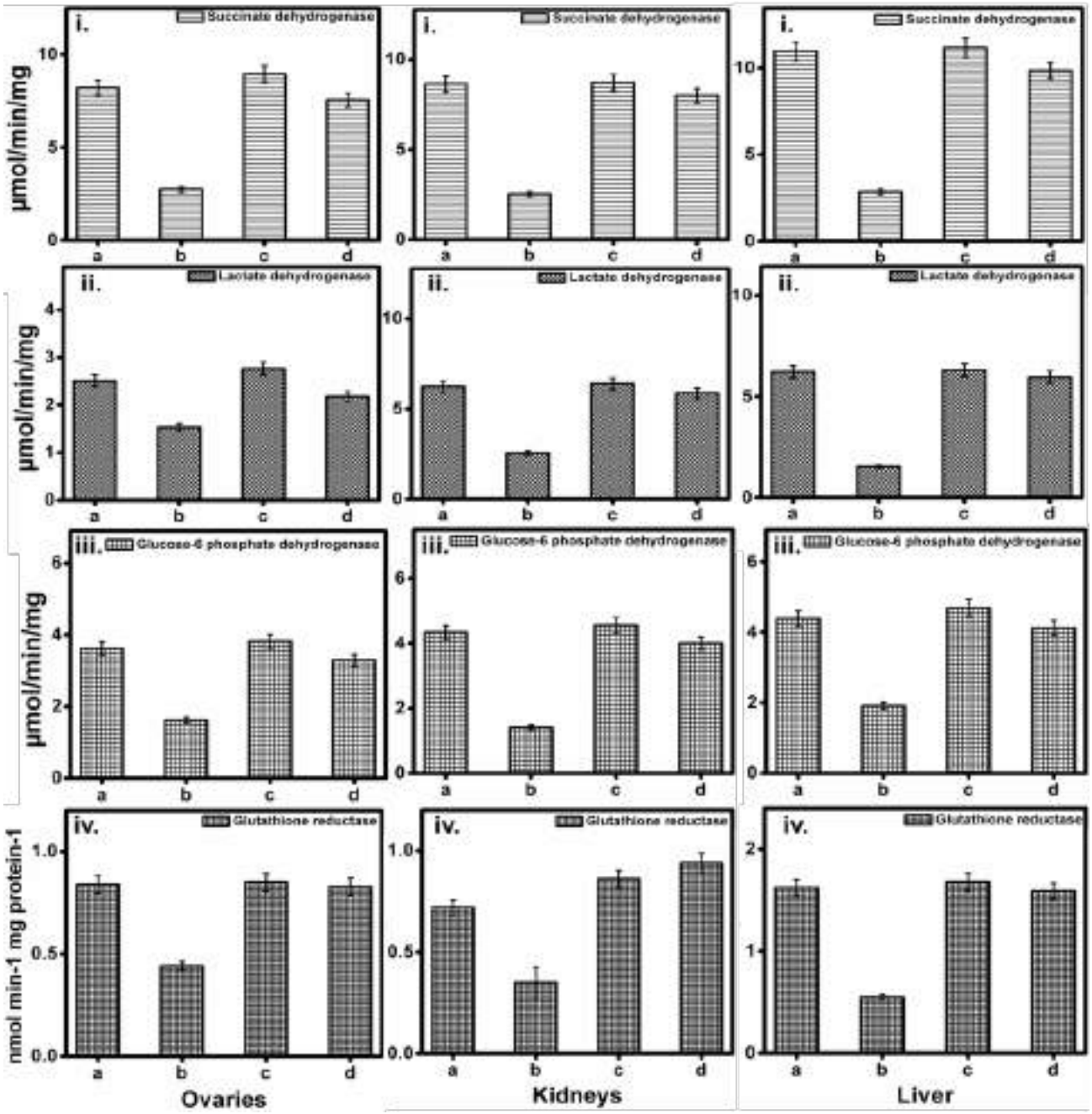

Figure. 11 Levels of redox enzymes i. Succinate dehydrogenase ii. Lactate dehydrogenase iii. glucose 6 phosphate dehydrogenase iv. Glutathione reductase in Ovaries, Kidneys, and Liver after treatment.

Assessment of antioxidant assays

The control group had CAT enzyme levels of 9.15, 10.85, and 12.85 µmol min-1 mg protein-1 in the ovaries, kidneys, and liver, respectively. Cisplatin treatment reduced levels to 4.58, 5.68, and 4.98 µmol min-1 mg protein-1, respectively. The probiotic group showed greater CAT levels (10.15, 11.15, and 13.15 µmol min-1 mg protein-1) when compared to the cisplatin group as represented in Figure. 12. The SOD enzyme levels in the ovaries, kidneys, and liver of the cisplatin group were decreased

3.81, 5.21, and 5.21 µmol min-1 mg protein-1 when compared with control 7.04, 8.44, and 9.44 µmol min-1 mg protein-1, probiotic (7.78, 8.78, and 9.78 µmol min-1 mg protein-1) and combination group 7.01, 8.65, and 9.35 µmol min-1 mg protein-1 respectively. In comparison to the control group (2.54, 1.51, and 1.51 nmol min-1 mg protein-1), probiotic group (1.45, and 1.45 nmol min-1 mg protein-1), and combination group (2.57, 1.75, and 1.75 nmol min-1 mg protein-1), the GPx enzyme levels in the ovaries, kidneys, and liver of the cisplatin group were decreased by 3.22, 2.62, and 2.32 nmol min-1 mg protein-1, respectively.

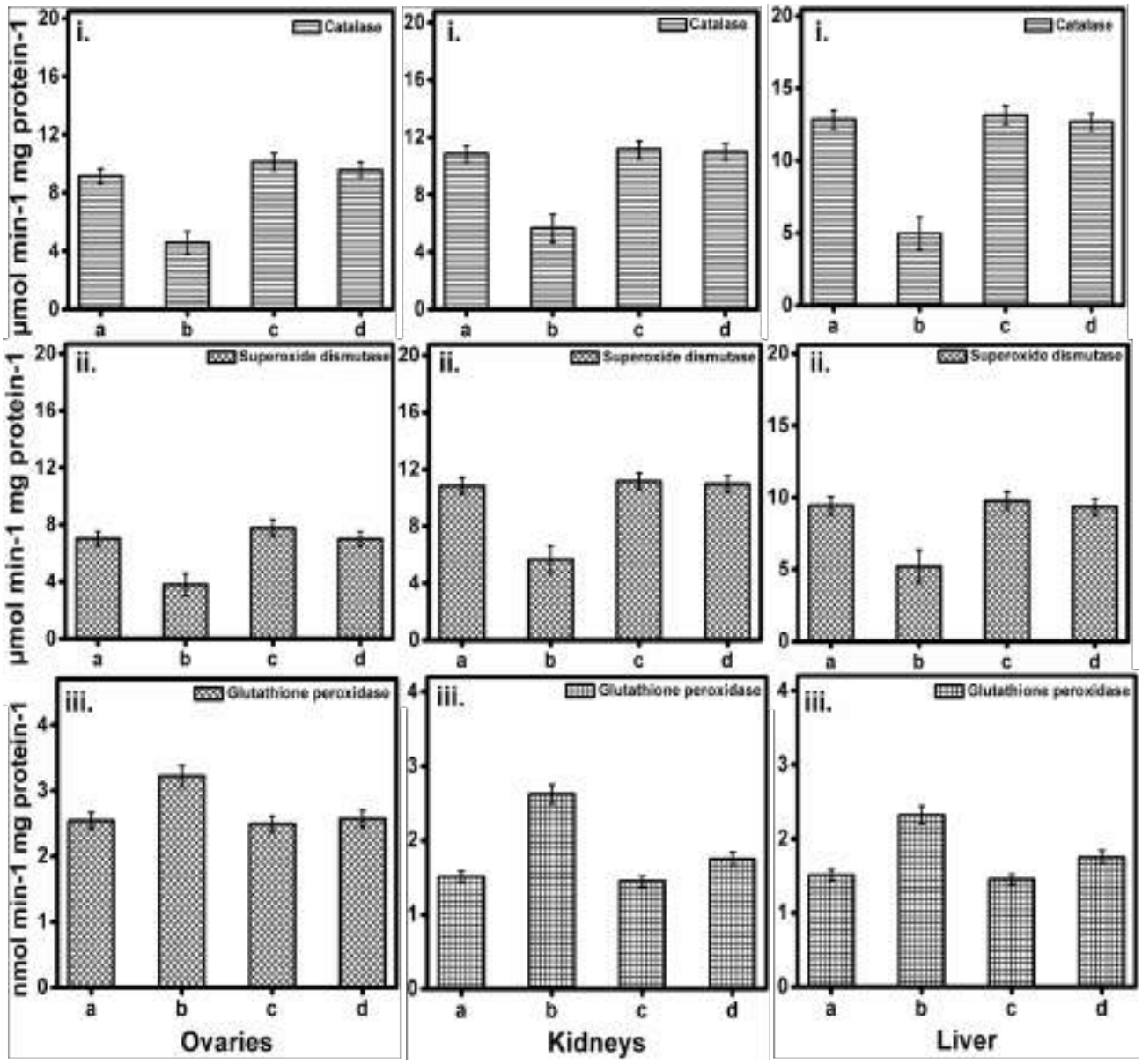

Figure. 12 Levels of antioxidant enzymes i. Catalase ii. Superoxide dismutase iii. Glutathione peroxidase in Ovaries, Kidneys, and Liver after treatment.

Estimation of oxidative stress

The amount of ROS in the cisplatin group increased in the ovaries, kidneys, and liver with 96.56, 89.25, and 91.99% inhibition, respectively, compared to 79.64, 67.14, and 80.09 % of inhibition in the probiotic group and control the ovaries (75.14), kidneys (68.64), the liver (79.12% of inhibition). However, when cisplatin and probiotics were combined, ROS levels were reduced 76.52 in the ovaries, 61.41 in the kidneys, and 77.6% of inhibition in the liver, compared to cisplatin alone as shown in Figure. 13. The control group's LPO levels were 10.29 in the kidneys, 11.49 in the ovaries, and 12.49 nmol MDA/g wt in the liver. The ovaries showed 15.83 nmol MDA/g wet wt, the kidneys 19.23 nmol MDA/g wet wt, and the liver 22.64 nmol MDA/g wet wt following cisplatin treatment. LPO levels in the probiotic group were 11.5 in the ovaries, 12.12 in the kidneys, and 12.52 nmol MDA/g wet wt in the liver. Probiotic treatment combined with cisplatin significantly decreased LPO levels to 10.85 in the ovaries, 11.98 in the kidneys, and 11.58 nmol MDA/g wet wt in the liver, suggesting that the probiotic was protecting against oxidative stress caused by cisplatin.

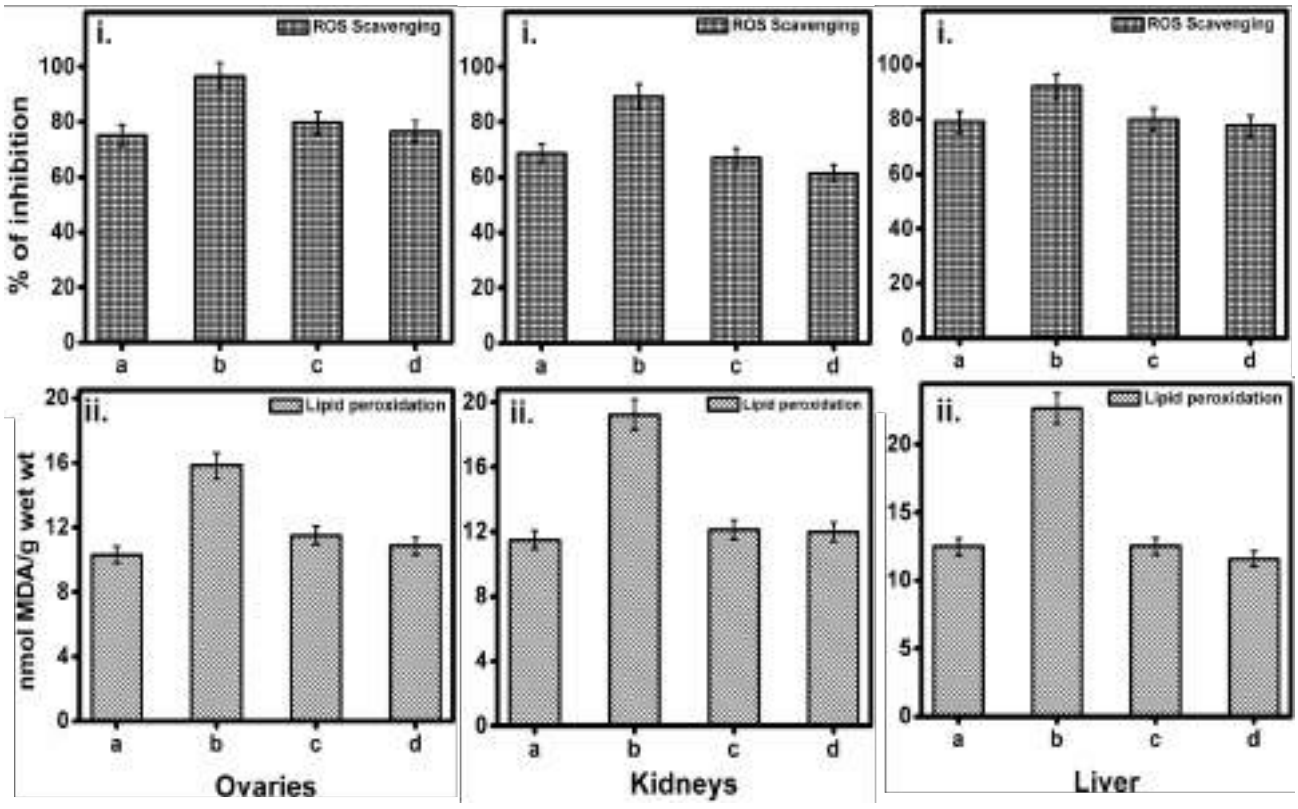

Figure. 13 Levels of oxidative stress through i. ROS scavenging activity ii. Lipid peroxidation in Ovaries, Kidneys, and Liver after treatment.

Determination of DNA damage by 8-OHdG and Caspase-3 Levels

The study investigated the impact of cisplatin and probiotics on the levels of 8-hydroxy-2'-deoxyguanosine (8-OHdG) and caspase-3 in different tissues. The results showed that there were significant variations between the treatment groups such as control, cisplatin, probiotics, and combination. The control group's 8-OHdG levels revealed 1.3 ng/mg DNA in the ovaries, 2.3 ng/mg DNA in the kidneys, and 1.8 ng/mg DNA in the liver; in contrast, the group treated with cisplatin had much higher amounts of these molecules in ovaries 3.9 ng/mg DNA, kidneys 5.7 ng/mg DNA, and liver 4.2 ng/mg DNA. The probiotic group yielded levels comparable to the control, with 1.4 ng/mg DNA in the ovaries, 2.1 ng/mg DNA in the kidneys, and 1.9 ng/mg DNA in the liver. In comparison to cisplatin alone, the combination of probiotics plus cisplatin decreased the 8-OHdG levels, demonstrating 2.3 ng/mg DNA in the ovaries, 3.0 ng/mg DNA in kidneys, and 2.5 ng/mg DNA in liver as depicted in Figure. 14. The cisplatin group showed an increase in caspase-3 activity of 45.2 U/mg in the ovaries, 50.8 U/mg in the kidneys, and 35.9 U/mg in the liver, compared to the control group's 25.1 U/mg in the ovaries, 20.3 U/mg in the kidneys, and 15.4 U/mg in the liver. Caspases-3 activity in the probiotic group was partially raised 30.5 U/mg in the ovaries, 22.4 U/mg in the kidneys, and 18.1 U/mg in the liver. When probiotics and cisplatin were combined, the results showed moderate levels of caspase-3 activity in the liver, kidneys, and ovaries (35.7 U/mg, 40.2 U/mg, and 28.6 U/mg, respectively). These findings imply that probiotics might lessen some of the cisplatin's oxidative and apoptotic effects in these tissues.

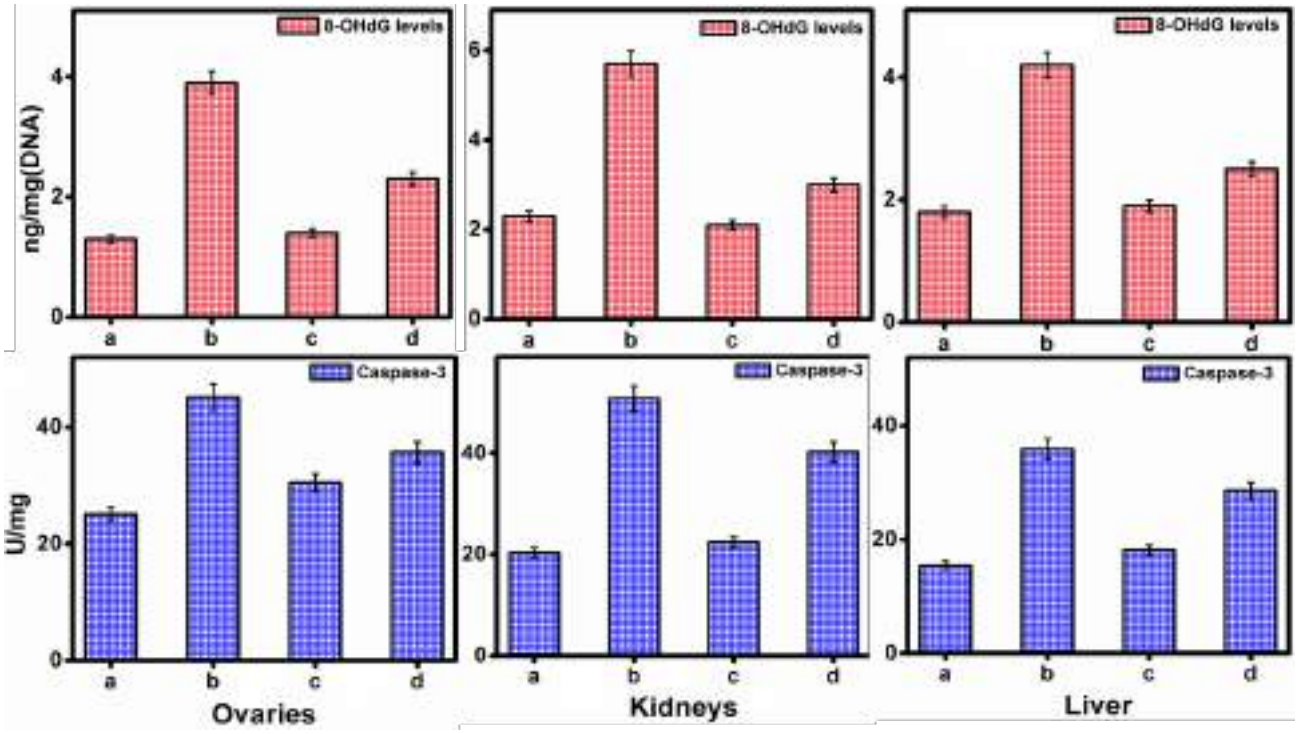

Figure. 14 Levels of DNA damage through 8-OHdG and caspase-3 in Ovaries, Kidneys, and Liver after treatment.

Histopathology

Significant variations between the treatment groups were found by histopathological evaluation of the ovarian tissues as represented in Figure. 15. The ovarian tissue in the control group had intact follicles and no damage to the cells, preserving normal architecture. On the other hand, the cisplatin-treated group showed significant cellular and structural damage as evidenced by follicular shrinkage, increased apoptotic bodies, and severe disruption of the ovarian stroma. The group that received only probiotics had ovarian tissues that closely mirrored the control group, with well-preserved follicles and no evidence of damage, indicating that probiotics had a preventive impact. The ovarian tissue in this combination group showed a substantial decrease in stromal disruption, fewer apoptotic bodies, and less follicular atrophy, indicating the restorative and protective benefits of probiotics in reducing ovarian damage caused by cisplatin. The kidney tissues in the control group showed no evidence of inflammation or injury and had a normal histological architecture with intact glomeruli and tubules. The cisplatin-treated group, on the other hand, showed notable histological damage, including

glomerular shrinkage, severe tubular necrosis, and interstitial inflammation. There was also significant tubular damage and extensive epithelial cell degeneration, which suggested that cisplatin was causing significant nephrotoxicity. The probiotic group had normal glomeruli and tubules as shown in Figure. 16. Surprisingly, compared to the cisplatin-only group, the histological analysis in the cisplatin + probiotic group showed a significant decrease in kidney damage with less necrosis and inflammation, and the tubular architecture was mostly intact, demonstrating the protective benefits of probiotics. The overall histological appearance was significantly more effective, despite the presence of small tubular degeneration. This indicates that probiotics have an ameliorating effect and can protect against cisplatin-induced nephrotoxicity. The liver tissues in the control group showed normal histological architecture, including clear sinusoidal gaps, well-preserved hepatic cells, and no indications of inflammation or necrosis. The liver tissues of the group treated with cisplatin exhibited significant damage, as evidenced by the presence of inflammatory cells, widespread necrosis, severe hepatocellular degeneration, and sinusoidal dilatation as shown in Figure. 17. These histological alterations indicate substantial cisplatin-induced liver damage. In comparison to the control group, the liver tissues of the probiotic-treated group showed slight histological changes. With occasional minor sinusoidal dilatation. When compared to the cisplatin group, the liver tissues in the cisplatin + probiotic therapy group exhibited a notable improvement. Significant restoration was observed, as evidenced by decreased hepatic degeneration, reduced necrosis, and low infiltration of inflammatory cells. Probiotics have been shown to have restorative and protective effects on liver damage caused by cisplatin, as seen by the apparent restoration of the liver architecture.

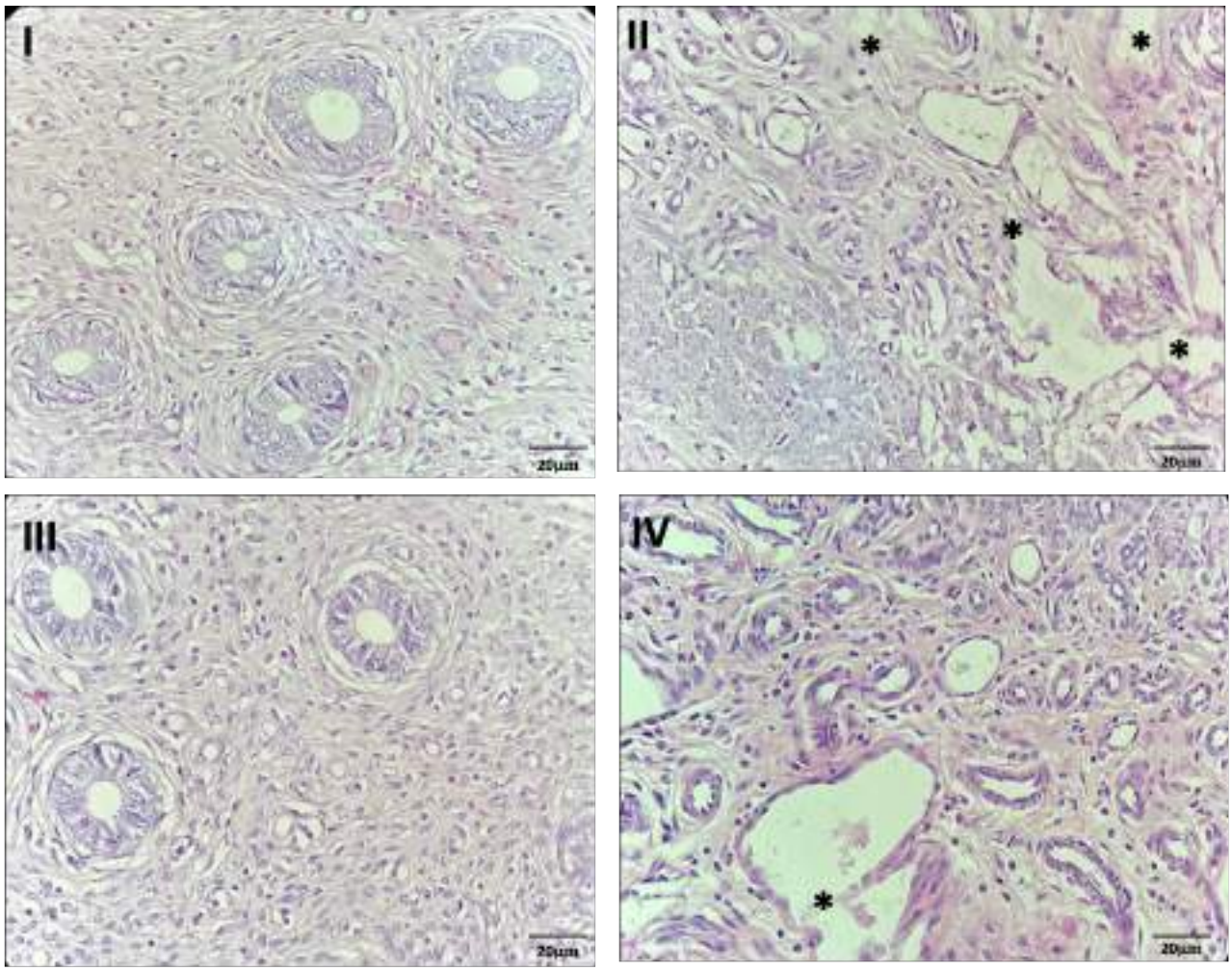

Figure. 15 The impact of cisplatin-induced toxicity in histopathology of ovaries of albino rats after treatment i) control, ii) cisplatin, iii) probiotic, iv) cisplatin + probiotic groups. (*) indicates the damage intended to that particular region.

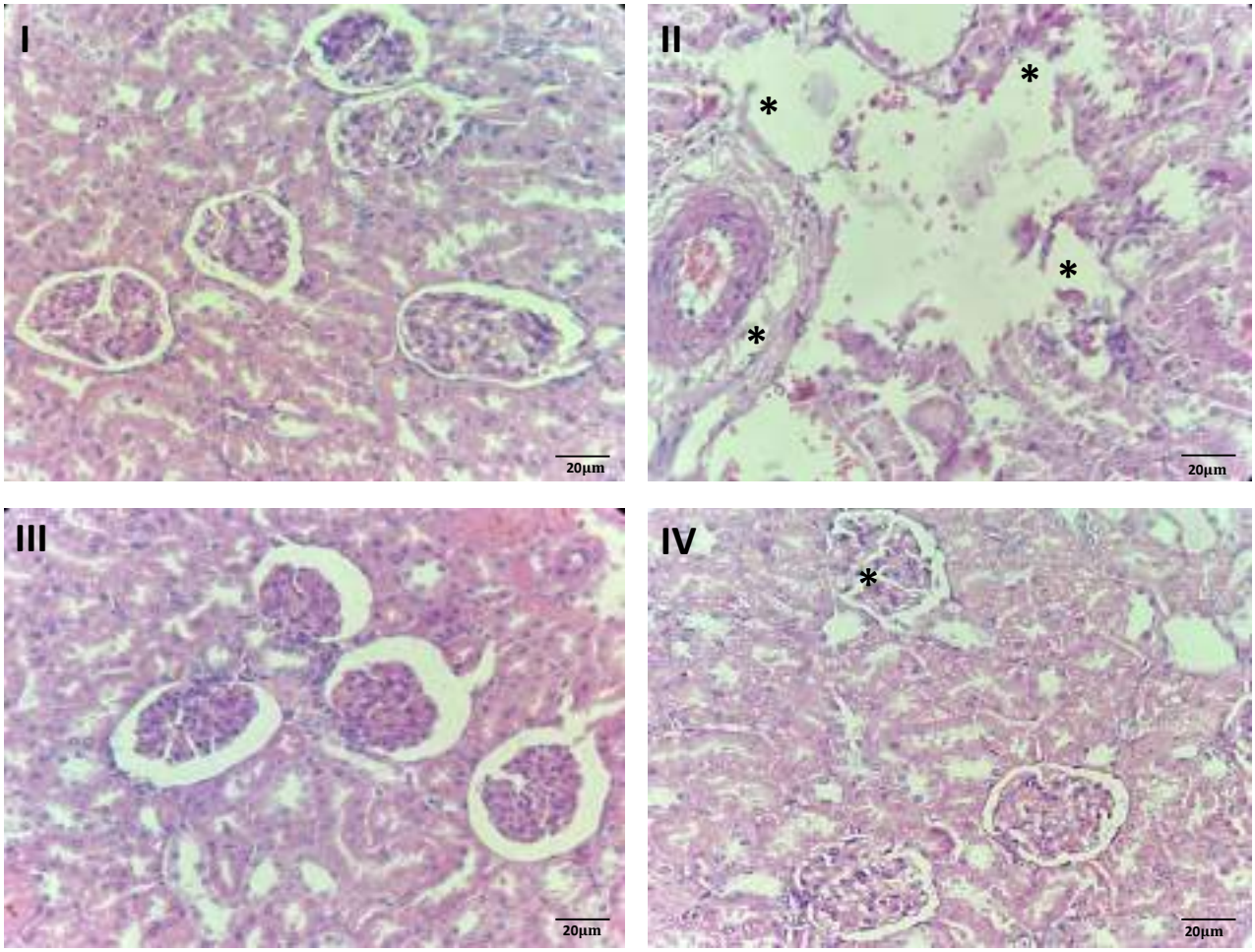

Figure. 16 The impact of cisplatin-induced toxicity in histopathology of kidneys of albino rats after treatment i) control, ii) cisplatin iii) probiotic,, iv) cisplatin + probiotic groups. (*) indicates the damage intended to that particular region.

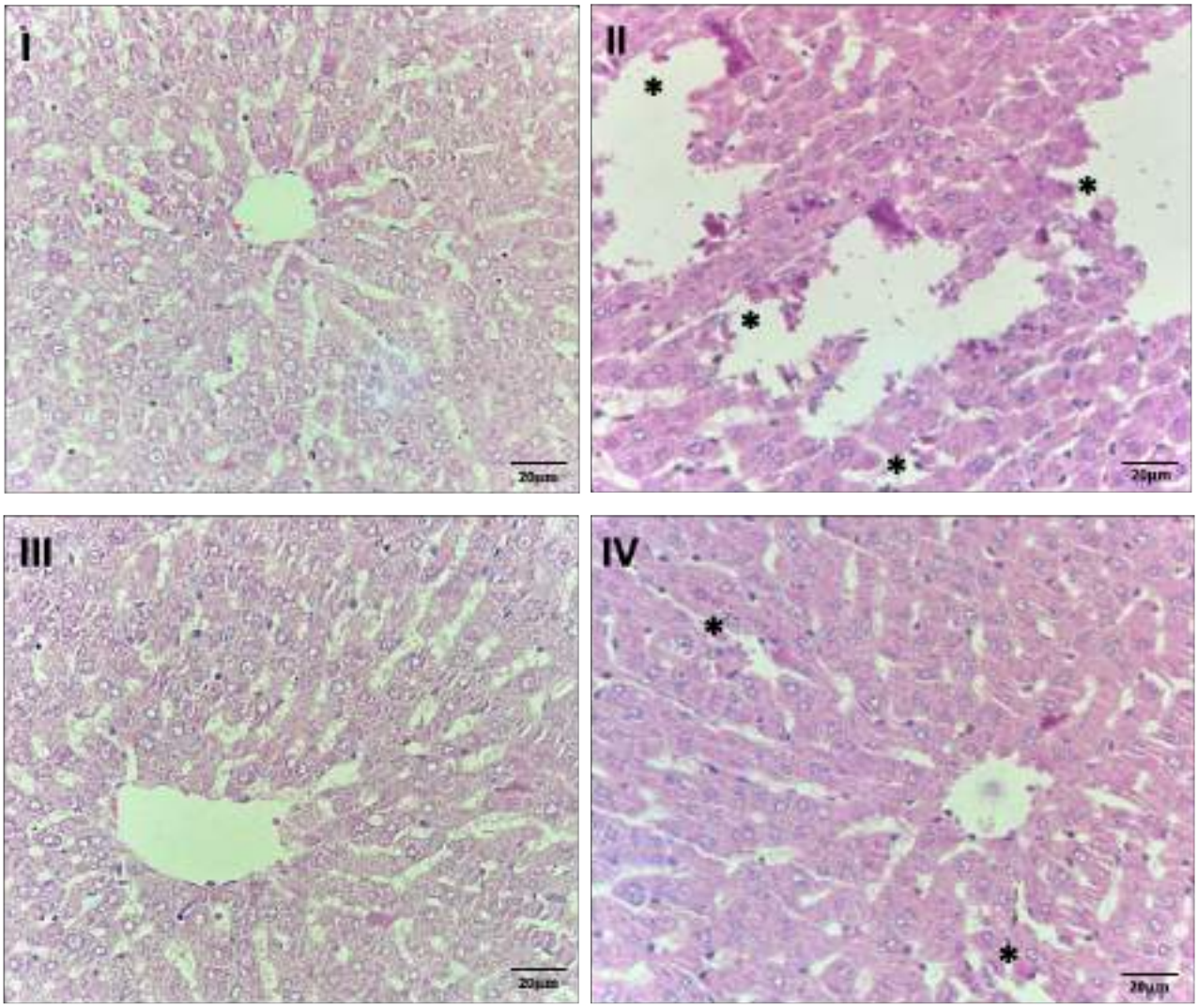

Figure. 17 The impact of cisplatin-induced toxicity in histopathology of liver of albino rats after treatment i) control, ii) cisplatin iii) probiotic,, iv) cisplatin + probiotic groups. (*) indicates the damage intended to that particular region.

6. Statistical analysis

All experimental data reported in the results are the means of triplicate measurements. The collected findings were evaluated and compared using Duncan's new Multiple range (DMR) test and reported differences were statistically significant at $P < 0.05$.

7. Discussion

The current study provides compelling evidence on the protective and restorative effects of probiotics

against the deleterious impacts of cisplatin. Our findings contribute to a growing body of literature that underscores the potential of probiotics in mitigating chemotherapy-induced toxicity, offering a promising adjunctive therapy to enhance patient outcomes. The significant reduction in SDH, LDH, G-6-PDH, and GR activities observed in the cisplatin-treated group across all examined tissues aligns with previous studies highlighting cisplatin's capability to induce oxidative stress and cellular damage. The drastic inhibition of these enzymes reflects cisplatin's interference with cellular metabolism and redox homeostasis, corroborating its cytotoxic mechanism of action. Intriguingly, the administration of probiotics alone not only maintained but, in some cases, enhanced the enzyme activities to levels comparable to or slightly higher than those in the control group. This suggests that probiotics bolster the intrinsic antioxidant defense mechanisms, potentially through upregulation of enzyme synthesis or stabilization of enzyme structures (Bustos et al., 2024). The combined treatment of cisplatin and probiotics resulted in intermediate enzyme activity levels, indicating a partial protective effect of probiotics. This partial restoration signifies that while probiotics cannot completely counteract the inhibitory effects of cisplatin, they can significantly attenuate the enzyme activity loss, thereby reducing oxidative stress and preserving cellular function to a notable extent.

The marked reduction in CAT, SOD, and GPx activities in the cisplatin-treated group underscores the extensive oxidative damage inflicted by cisplatin. These enzymes are crucial in detoxifying ROS and their diminished activity corroborates the elevated oxidative stress and subsequent cellular injury (Sultana et al., 2012). Probiotic treatment alone elevated the activities of these antioxidant enzymes beyond control levels, suggesting that probiotics may upregulate the expression or activity of these crucial enzymes, enhancing the cellular antioxidant capacity. The combined treatment group exhibited enzyme activities that were higher than those in the cisplatin group but lower than the probiotic group alone, reinforcing the idea that probiotics confer a significant protective effect against cisplatin-induced oxidative damage. This partial restoration of antioxidant enzyme activities points to the potential of probiotics to mitigate chemotherapy-induced oxidative stress, offering a non-invasive strategy to improve patient resilience to chemotherapeutic regimens. The increased ROS levels and LPO in the cisplatin group, as evidenced by higher inhibition percentages and MDA levels,

respectively, highlight the oxidative assault exerted by cisplatin on cellular components. Elevated ROS and LPO are hallmarks of oxidative stress, leading to membrane damage, protein oxidation, and DNA damage (Demirci-Cekic et al., 2022). Probiotic treatment significantly reduced ROS levels and LPO, indicating its potent antioxidative properties. This reduction suggests that probiotics may enhance the scavenging of ROS and inhibit lipid peroxidation, thereby protecting cellular integrity. The combined treatment further demonstrated reduced ROS levels and LPO compared to the cisplatin group, although not as low as the probiotic group alone. This intermediate effect suggests that probiotics can buffer the oxidative impact of cisplatin, reducing overall cellular damage and preserving tissue function. These findings are particularly relevant for clinical settings where reducing oxidative stress can enhance patient tolerance to chemotherapy and potentially improve treatment outcomes.

The elevated levels of 8-OHdG and caspase-3 activity in the cisplatin-treated group reflect significant DNA damage and apoptosis, consistent with cisplatin's known genotoxic and pro-apoptotic effects. These biomarkers are critical indicators of oxidative DNA damage and programmed cell death, respectively (Rahman et al., 2023). The probiotic group showed levels comparable to the control, suggesting that probiotics can effectively prevent cisplatin-induced genotoxicity and apoptosis. This protective effect is likely mediated through the reduction of oxidative stress and enhancement of DNA repair mechanisms. The combined treatment group exhibited lower 8-OHdG levels and caspase-3 activity compared to the cisplatin group, indicating that probiotics mitigate DNA damage and apoptosis induced by cisplatin. This partial protection implies that probiotics may enhance DNA repair pathways and inhibit apoptotic signaling, thereby preserving cellular viability. These findings highlight the potential of probiotics to reduce chemotherapy-induced genotoxicity and improve cellular resilience, which could translate to better clinical outcomes for patients undergoing chemotherapy. Histopathological analysis revealed significant tissue damage in the cisplatin-treated group, characterized by structural disintegration, increased apoptotic bodies, and extensive cellular necrosis in the ovaries, kidneys, and liver. These pathological changes are indicative of severe cytotoxicity and tissue dysfunction induced by cisplatin. In contrast, the probiotic group showed tissue

architecture like the control, with minimal damage, suggesting a protective effect of probiotics against cisplatin-induced histopathological changes. The combined treatment group displayed a significant reduction in tissue damage compared to the cisplatin group, with less necrosis, inflammation, and structural disruption. This histological improvement underscores the protective role of probiotics in preserving tissue integrity and function. The observed restoration of tissue architecture in the combined treatment group suggests that probiotics can ameliorate cisplatin-induced histopathological damage, potentially through anti-inflammatory and anti-apoptotic mechanisms.

Conclusion

The cumulative evidence from this study indicates that probiotics confer significant protective and restorative effects against cisplatin-induced oxidative stress, enzymatic inhibition, DNA damage, and tissue injury. The partial restoration of redox enzyme activities, antioxidant capacity, and histological integrity in the combined treatment group suggests that probiotics can mitigate the adverse effects of cisplatin, improving overall tissue resilience and function. These findings advocate for the inclusion of probiotics as an adjunctive therapy in chemotherapy regimens to enhance patient outcomes and reduce chemotherapy-induced toxicity. Further clinical studies are warranted to validate these findings and elucidate the underlying mechanisms of probiotic action in mitigating chemotherapeutic side effects.

8. Impact of the research in the advancement of knowledge or benefit to mankind

Benefits to Mankind:

Safer Chemotherapy: Cisplatin is a widely used chemotherapy drug, but its toxicity can be severe, leading to side effects that affect the patient's quality of life. If probiotics are shown to mitigate this toxicity, it could lead to safer chemotherapy regimens, allowing patients to undergo treatment with fewer adverse effects.

Personalized Medicine: Understanding the role of probiotics in reducing cisplatin toxicity could contribute to personalized medicine approaches. By considering an individual's gut microbiome

composition, doctors could tailor probiotic interventions to enhance the patient's ability to tolerate cisplatin treatment.

Reduced Healthcare Costs: Severe toxicity from cisplatin often requires additional medical interventions and care, increasing healthcare costs. If probiotics can mitigate these toxic effects, it could potentially reduce the need for extensive medical interventions, leading to cost savings for both patients and healthcare systems.

Improved Treatment Compliance: The fear of side effects often leads to reduced patient compliance with chemotherapy. If probiotics can reduce the severity of cisplatin-induced side effects, patients might be more likely to complete their treatment regimens, thus improving treatment outcomes.

Enhanced Quality of Life: Minimizing the adverse effects of cisplatin can greatly improve the quality of life for cancer patients undergoing treatment. Reduced toxicity could mean less pain, nausea, and discomfort, allowing patients to maintain a better overall well-being during their treatment journey.

In summary, the research on using probiotics to reduce cisplatin-induced toxicity in albino rats not only has the potential to advance our understanding of toxicology and microbiome-host interactions but also holds promise for improving cancer treatment outcomes and patients' quality of life.

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