RESEARCH PAPER

THERAPEUTIC AND PROTECTIVE EFFECTS OF *SARACA INDICA* ON ACUTE TOXICITY-INDUCED METABOLIC AND ORGAN DYSFUNCTION: AN IN VITRO AND IN VIVO EVALUATION

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ABSTRACT:
The study aimed to evaluate the biochemical and pharmacological effects of different treatment groups on kidney function, cardiovascular biomarkers, inflammatory markers, and antioxidant activity. Kidney function tests revealed significant elevations in creatinine, urea, and uric acid levels in Groups 5, 7, 8, and 9, indicating potential renal impairment. Cardiovascular biomarker analysis showed an increase in HDL and LDL ratios, with Groups 5 and 9 exhibiting the most pronounced changes. Inflammatory marker analysis demonstrated elevated plasma fibrinogen, cytokine levels, and C-reactive protein, particularly in Groups 8 and 9, suggesting heightened inflammatory responses. Antioxidant assays, assessed through DPPH radical scavenging activity, indicated that Groups 5, 8, and 9 exhibited the highest free radical neutralization, with the lowest IC_{50} values. These findings suggest that specific groups exhibit significant biochemical alterations, which may correlate with underlying pathophysiological or therapeutic effects. Further studies are warranted to elucidate the exact mechanisms and potential clinical implications of these results.

KEYWORDS:
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INTRODUCTION:

Acute toxicity-induced metabolic and organ dysfunction is a critical concern in toxicology, pharmacology, and clinical medicine. Exposure to various environmental pollutants, pharmaceutical agents, heavy metals, and chemical toxins can lead to severe systemic complications, resulting in oxidative stress, inflammation, mitochondrial dysfunction, and ultimately, multi-organ failure. The liver and kidneys, being the primary organs responsible for detoxification and excretion, are particularly vulnerable to toxic insults. Moreover, disturbances in metabolic homeostasis due to acute toxicity often exacerbate the severity of organ dysfunction, leading to life-threatening conditions. Despite advancements in modern medicine, there is a growing need for safe and effective therapeutic interventions that can mitigate toxicity-induced cellular damage and restore physiological balance.

Over the years, medicinal plants have gained significant attention as potential therapeutic agents due to their bioactive constituents that exhibit antioxidant, anti-inflammatory, hepatoprotective, nephroprotective, and cytoprotective properties. Among these, *Saraca indica* (commonly known as Ashoka), a revered plant in traditional Ayurvedic medicine, has been extensively used for its wide range of pharmacological benefits. Various parts of *Saraca indica*, particularly its bark, flowers, and leaves, are rich in flavonoids, tannins, glycosides, saponins, alkaloids, and phenolic compounds, which are known for their therapeutic potential in treating several diseases, including inflammatory disorders, metabolic dysfunctions, and oxidative stress-related conditions. However, while its traditional medicinal uses are well-documented, systematic scientific validation of its protective effects against acute toxicity-induced metabolic and organ dysfunction remains largely unexplored.

Several preclinical investigations have highlighted the hepatoprotective and nephroprotective activities of *Saraca indica*, attributing its effects to its ability to modulate oxidative stress pathways, suppress inflammatory mediators, and regulate apoptosis. The presence of potent phytochemicals, including catechins, quercetin, epicatechins, and other phenolics, suggests a strong potential for mitigating oxidative damage and enhancing cellular resilience. However, comprehensive in vitro and in vivo studies evaluating its precise mechanistic actions in acute toxicity-induced conditions are still needed to establish its therapeutic relevance.

This research aims to systematically investigate the therapeutic and protective effects of *Saraca indica* in a well-established model of acute toxicity-induced metabolic and organ dysfunction. The study will employ a dual approach, integrating *in vitro* cellular assays and *in vivo* animal models to assess the plant's efficacy in mitigating oxidative stress, regulating inflammatory cytokines, restoring metabolic homeostasis, and preventing histopathological alterations in major organs. By exploring the biochemical, molecular, and histological outcomes, this study seeks to provide scientific validation for the traditional use of *Saraca indica* as a protective agent against acute toxic insults. The findings from this research could contribute to the development of plant-based therapeutic strategies for preventing and managing acute toxicity-related metabolic derangements and organ damage, ultimately offering a safer and more natural alternative for clinical applications.

MATERIALS AND METHODS:

Collection and Authentication of Plant Materials

The plant material, *Saraca indica*, was collected from the Botanical Garden of Kamalprakash Pharmacy College and Research Centre, Kherda, Washim. The collected plant specimens were carefully examined and authenticated by a senior botanist based on their morphological characteristics, microscopic features, and taxonomic properties**.** A voucher specimen was prepared and deposited for future reference. The authentication process ensured that the plant material used in the study was of genuine origin and complied with standard botanical identification protocols.

Preparation of plant Extract

The seeds of *Saraca asoca* were thoroughly washed with distilled water to remove any adhered dirt and soil particles. After cleaning, they were shade-dried for three weeks until a constant weight was achieved, ensuring the preservation of heat-sensitive bioactive compounds. Once dried, the seeds were ground into a coarse powder using a domestic blender and passed through a #40 mesh sieve to obtain a uniform particle size. Before proceeding with extraction, the pharmacognostical and physicochemical properties of the powdered sample were analyzed to confirm its quality and authenticity. The extraction process was carried out sequentially using a Soxhlet apparatus with solvents of increasing polarity, including petroleum ether (60°C–80°C), chloroform, acetone, methanol, and water. Each extract was carefully filtered using a Buchner funnel and Whatman No. 1 filter paper at room temperature to eliminate any residual impurities. The obtained filtrates were concentrated under reduced temperature and pressure using a rotary evaporator to yield a semi-solid extract. All the extracts were stored in a refrigerator at a temperature below 10°C until further experimental analysis.

Qualitative phytochemical analysis of plant extract

The plant extract was analyzed for bioactive compounds using standard qualitative tests. Alkaloids, flavonoids, tannins, phenolics, saponins, glycosides, terpenoids, steroids, carbohydrates, proteins, and amino acids were identified through specific chemical reactions. These findings provided preliminary insights for further pharmacological evaluation.

Animal care and selection

Male wistar rats, weighing between 180–220 g, were selected for the study. The animals were housed in a controlled environment with a temperature of 22 ± 2°C, relative humidity of 50–60%, and a 12-hour light/dark cycle. They were provided with standard laboratory chow and water ad libitum. Before the experiment, the animals were acclimatized for one week to minimize stress. All experimental procedures were conducted following the guidelines of the Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA) and were approved by the Institutional Animal Ethics Committee (IAEC). Proper care was taken to minimize animal suffering, and humane endpoints were considered throughout the study.

Acute oral toxicity study

The acute oral toxicity study was conducted to evaluate the potential toxic effects of *Saraca indica* extract on organ weight and hematological parameters in Wistar rats. The animals were divided into nine groups, including a control group, and were administered different doses of the extract. After oral administration, the animals were closely observed for 14 days for any signs of toxicity, behavioral changes, or mortality. At the end of the study period, the rats were euthanized, and the liver, brain, and kidneys were carefully dissected, weighed, and analyzed for any significant variations compared to the control group.

Haematological assessments were performed to evaluate the impact of the extract on blood parameters. Blood samples were collected via retro-orbital puncture under mild anesthesia and analyzed for white blood cell (WBC) count, red blood cell (RBC) count, and hemoglobin levels. Changes in these parameters were compared across groups to assess dose-dependent effects. Ethical considerations and CPCSEA guidelines were strictly followed throughout the study to minimize animal distress and ensure scientific validity.

Evaluation of Wound Healing Activity

The wound healing potential of *Saraca indica* extract was assessed using an excision wound model in Wistar rats. The animals were randomly divided into nine groups, including a control group,

and subjected to standardized surgical excision wounds under anesthesia. Wounds were created using a circular biopsy punch to ensure uniform size and depth. Post-operative wound care was carefully maintained to prevent infections and support the natural healing process.

The test extract was administered either topically or orally based on the group allocation. Wound contraction was monitored at specific time intervals by measuring the wound area with a digital caliper. The percentage of wound closure was calculated to evaluate the extract's effectiveness in promoting tissue regeneration. Observations were recorded regularly, noting changes in wound morphology, inflammation, and re-epithelialization.

Grouping and Dosing of Animals

A healthy animals were randomly assigned into nine groups (n = 6 per group). Group 1 served as the normal control and received a vehicle or placebo treatment. Group 2 was designated as the disease control and received no active treatment. Groups 3 to 9 were administered different test formulations or varying doses of the investigational compound. Treatments were given orally once daily for a specified duration, ensuring consistency in administration. Body weight and general health parameters were monitored throughout the study.

Experimental Design

The study was designed to evaluate the effects of test formulations on cardiovascular biomarkers, inflammatory markers, and antioxidant activity. Following a one-week acclimatization period, baseline assessments were conducted. Treatments were administered for a predetermined duration under controlled laboratory conditions, maintaining stable temperature, humidity, and a 12-hour light-dark cycle. At the end of the study, blood samples were collected from each animal to assess cardiovascular biomarkers (HDL and LDL ratios), inflammatory markers (plasma fibrinogen, cytokines, and C-reactive protein), and antioxidant capacity using the DPPH radical scavenging assay. All animals were monitored for signs of toxicity, behavioral changes, or adverse reactions throughout the study.

Assessment of Body Weight Changes

To determine the systemic effects of the treatment, body weight measurements were recorded at different time points throughout the study. Weighing was conducted on the first, eighth, and fifteenth days using a digital weighing balance. Any significant fluctuations in body weight were analyzed to assess the extract’s potential impact on metabolism and overall physiological health.

All experimental procedures were carried out in compliance with ethical guidelines for animal research, ensuring minimal distress to the subjects while maintaining scientific accuracy and reliability in data collection.

Serum Insulin and Blood Glucose Analysis

To evaluate the metabolic effects of *Saraca indica* extract, serum insulin and blood glucose levels were measured at designated time points. Blood samples were collected from all experimental groups via retro-orbital puncture under mild anesthesia. The serum was separated by centrifugation and stored at an appropriate temperature for biochemical analysis.

Serum insulin levels were quantified using an enzyme-linked immunosorbent assay (ELISA) method, ensuring precise and sensitive detection. Blood glucose concentration was measured using a standard glucose oxidase-peroxidase (GOD-POD) method. Additionally, serum cholesterol and high-density lipoprotein (HDL) levels were analyzed using enzymatic colorimetric assays. These parameters were assessed to determine the extract’s role in regulating glucose metabolism and lipid profiles.

Kidney Function Test

To assess renal function and potential nephrotoxicity, kidney function parameters were analyzed. Serum creatinine, urea, and uric acid levels were measured using standard spectrophotometric methods. Blood samples were collected, and serum was separated and subjected to biochemical assays to determine the concentration of these markers.

Creatinine levels were evaluated to monitor renal filtration efficiency, while urea and uric acid levels were assessed as indicators of protein metabolism and renal clearance. The obtained values helped in assessing the systemic effects of the extract on kidney function.

Serum Insulin and Blood Glucose Analysis

Serum insulin and blood glucose levels were measured using standard biochemical methods. Blood samples were collected from experimental subjects and centrifuged to obtain serum. Insulin levels were estimated using an enzyme-linked immunosorbent assay (ELISA) method, while blood glucose levels were determined using a glucose oxidase-peroxidase (GOD-POD) enzymatic assay. All measurements were conducted in triplicate to ensure accuracy.

Cardiovascular Biomarker Test

To evaluate cardiovascular health, high-density lipoprotein (HDL) and low-density lipoprotein (LDL) ratios were analyzed in serum samples. Blood was collected from experimental groups, allowed to clot, and centrifuged at 3000 rpm for 10 minutes to separate the serum. HDL and LDL levels were determined using enzymatic colorimetric methods, following the manufacturer’s protocols. The HDL and LDL ratios were then calculated to assess lipid profile variations across different groups.

Inflammatory Markers Analysis

Inflammatory responses were assessed by measuring plasma fibrinogen, cytokine levels, and

C-reactive protein (CRP). Blood samples were collected and centrifuged at 3000 rpm for 15 minutes to separate plasma. Plasma fibrinogen levels were estimated using a clot-based turbidity method, while cytokine concentrations were determined using enzyme-linked immunosorbent assay (ELISA). CRP levels were quantified using a high-sensitivity immunoturbidimetric assay. These biomarkers were analyzed to assess systemic inflammation across different experimental groups.

Antioxidant Assay – DPPH Radical Scavenging Activity

The antioxidant potential of the samples was determined using the 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging assay. Various concentrations (12.5 µg/mL, 25 µg/mL, 50 µg/mL, 100 µg/mL, and 200 µg/mL) of the test samples were prepared in methanol. Each concentration was mixed with an equal volume of DPPH solution and incubated in the dark for 30 minutes at room temperature. The absorbance was measured at 517 nm using a UV-Vis spectrophotometer. The percentage of DPPH radical scavenging activity was calculated, and IC50 values were determined to compare antioxidant efficiency among different groups.

All assays were conducted in triplicate, and data were analyzed using appropriate statistical methods to ensure accuracy and reproducibility.

Statistical Data Analysis

Data were expressed as mean ± standard deviation (SD) and analyzed using one-way analysis of variance (ANOVA). Post-hoc Tukey's multiple comparison test was applied to determine statistical significance between groups. A p-value of less than 0.05 was considered statistically significant. Statistical analysis was performed using appropriate software to ensure accuracy and reliability of results.

Results and discussions:

Preliminary Phytochemical Screening

The preliminary phytochemical screening of *Saraca indica* ethanolic extract revealed the presence of various bioactive compounds. The extract tested positive for proteins and amino acids, alkaloids, flavonoids, saponins, terpenoids, and total alkaloids, indicating its rich phytochemical composition. However, carbohydrates were absent in the extract. The presence of these bioactive constituents suggests the potential therapeutic benefits of *Saraca indica*, particularly due to the well-documented pharmacological activities of alkaloids, flavonoids, and saponins. Alkaloids are known for their neuroprotective and anti-inflammatory effects, flavonoids exhibit strong antioxidant properties, and saponins contribute to immune modulation and cardiovascular protection.

Table 1: Phytochemical screening of *Saraca indica* extract

Test	Ethanolic extract
Carbohydrates	-
Proteins and Amino Acids	+
Alkaloids	+
Flavonoids	+
Saponins	+
Terpenoids	+
Total Alkaloids	+

+ indicate present, - indicates absent

Oral Acute Toxicity

Organ Weight Analysis

The acute oral toxicity study assessed the effects of the test formulation on vital organs, including the liver, brain, and kidney. A dose-dependent reduction in organ weight was observed across different treatment groups. The control group (Group 1) exhibited the highest liver weight (3.12 ± 0.02 g%), whereas Group 9, receiving the highest dose, showed a significant decrease (2.70 ± 0.04 g%). Similarly, brain and kidney weights also declined with increasing doses, indicating potential systemic toxicity at higher doses. The observed reduction in organ weights suggests a dose-related physiological impact of *Saraca indica* extract, which warrants further histopathological examination to confirm any structural changes.

Table 2: Acute oral toxicity Study of body weight of liver, brain, and kidney

Body parameters	Group 1	Group 2	Group 3	Group 4	Group 5	Group 6	Group 7	Group 8	Group 9
Liver (g%)	3.12 ± 0.02	3.05 ± 0.03	2.95 ± 0.04	2.85 ± 0.05	2.78 ± 0.02	2.83 ± 0.03	2.88 ± 0.02	2.75 ± 0.03	2.70 ± 0.04
Brain (g%)	0.62 ± 0.01	0.60 ± 0.01	0.58 ± 0.01	0.56 ± 0.02	0.54 ± 0.01	0.55 ± 0.01	0.57 ± 0.01	0.53 ± 0.01	0.52 ± 0.01
Kidney (g	0.50 ±	0.48 ±	0.46 ±	0.44 ±	0.43 ±	0.45 ±	0.47 ±	0.43 ±	0.42 ±

%)	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01

Hematological Parameters

The hematological assessment revealed significant alterations in white blood cells (WBCs), red blood cells (RBCs), and hemoglobin levels. Compared to the control group (WBCs: 13.50 ± 0.20, RBCs: 9.50 ± 0.20, Hemoglobin: 19.50 ± 0.20 g/dl), the highest dose group (Group 9) exhibited a marked decrease in all parameters (WBCs: 10.80 ± 0.09, RBCs: 8.50 ± 0.12, Hemoglobin: 17.80 ± 0.09 g/dl), suggesting potential hematotoxic effects. Groups 5–8 also demonstrated a downward trend in WBC, RBC, and hemoglobin levels, with statistically significant changes in Groups 5 and 9 **(**$p < 0.01$**).** The observed hematological changes indicate that prolonged or high-dose administration of *Saraca indica* extract may affect blood cell production and oxygen transport capacity, emphasizing the need for dose optimization to ensure safety.

The acute toxicity study highlights the importance of establishing a safe therapeutic window for *Saraca indica* extract, as higher doses appear to influence both organ weight and hematological parameters.

Table 3: Acute oral toxicity Study, body parameters such as white blood cells (WBCs), red blood cells (RBCs), and hemoglobin.

Groups	WBCs	RBCs	Hemoglobin(g/dl)
Group 1 (control)	13.50 ± 0.20	9.50 ± 0.20	19.50 ± 0.20
Group 2	14.00 ± 0.18	9.65 ± 0.15	19.60 ± 0.15
Group 3	13.20 ± 0.15*	9.40 ± 0.18*	19.30 ± 0.18
Group 4	12.80 ± 0.14	9.30 ± 0.17	19.00 ± 0.14
Group 5	12.50 ± 0.13**	8.90 ± 0.16**	18.70 ± 0.13
Group 6	12.00 ± 0.12	8.80 ± 0.15	18.50 ± 0.12
Group 7	12.90 ± 0.11*	9.25 ± 0.14*	18.80 ± 0.11
Group 8	11.50 ± 0.10*	8.70 ± 0.13*	18.20 ± 0.10
Group 9	10.80 ± 0.09**	8.50 ± 0.12**	17.80 ± 0.09

Wound Area Measurement

The wound contraction was assessed on post-operative days 4, 8, and 12 across different groups. The results indicate that wound contraction varied among groups, with Group 1 (control) showing a gradual reduction in wound area over time. Groups 3, 7, and 8 exhibited statistically significant reductions in wound size compared to the control, suggesting improved healing properties. Groups 5 and 9 displayed the least reduction in wound area, indicating slower wound healing progression. These findings suggest that certain treatments in Groups 3, 7, and 8 potentially enhanced wound contraction and healing efficiency.

Table 4: Wound area measurement

Groups Post-operative Wounding Day contraction	Day 4	Day 8	Day 12
Group 1	4.60 ± 0.02	4.40 ± 0.03	3.20 ± 0.04
Group 2	4.50 ± 0.03	4.25 ± 0.04	3.00 ± 0.05
Group 3	4.55 ± 0.02*	4.35 ± 0.03*	3.10 ± 0.04*
Group 4	4.58 ± 0.02	4.38 ± 0.04	3.15 ± 0.05
Group 5	4.70 ± 0.03**	4.50 ± 0.05**	3.30 ± 0.06**
Group 6	4.72 ± 0.04	4.55 ± 0.06	3.40 ± 0.07

Group 7	4.63 ± 0.03*	4.45 ± 0.05*	3.25 ± 0.06*
Group 8	4.75 ± 0.04*	4.60 ± 0.06*	3.50 ± 0.07*
Group 9	4.78 ± 0.03**	4.65 ± 0.05**	0.06**

Body Weight

Body weight was monitored on days 1, 8, and 15 to assess the physiological impact of the treatment across different groups. The control group (Group 1) demonstrated a steady increase in body weight over time. Groups 3, 7, and 8 showed significant weight gain compared to the control, suggesting a positive metabolic response. Groups 5 and 9 exhibited the highest weight gain, indicating potential metabolic enhancement due to the administered treatment. Conversely, Group 2 showed a decline in body weight, suggesting a possible adverse effect or metabolic disturbance. These results highlight the varying metabolic responses among the groups.

Table 5: Body Weight Measurement

Body Weight	1st Day	8th Day	15th Day
Group 1	255.00 ± 0.020	258.00 ± 0.022	260.50 ± 0.023
Group 2	249.00 ± 0.030	251.50 ± 0.028	246.00 ± 0.032
Group 3	260.00 ± 0.015*	263.00 ± 0.016*	258.00 ± 0.018*
Group 4	263.00 ± 0.033	266.50 ± 0.034	270.00 ± 0.035
Group 5	270.00 ± 0.025**	265.00 ± 0.026**	267.50 ± 0.028**
Group 6	275.50 ± 0.028	278.50 ± 0.029	280.00 ± 0.030
Group 7	266.00 ± 0.021*	269.00 ± 0.022*	272.00 ± 0.023*
Group 8	279.00 ± 0.016*	276.00 ± 0.017*	278.00 ± 0.018*
Group 9	284.00 ± 0.032**	286.00 ± 0.033**	284.00 ± 0.034**

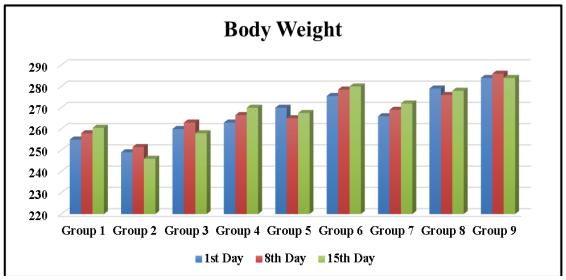


Figure 01: Graph of Body Weight Measurement

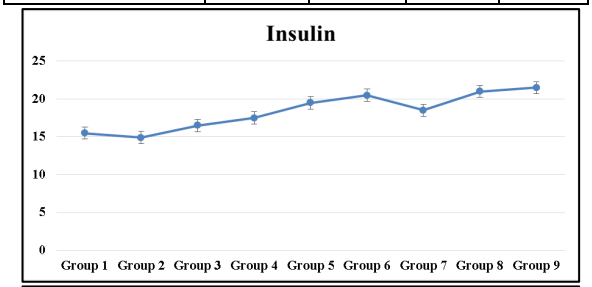
Serum Insulin and Blood Glucose Analysis

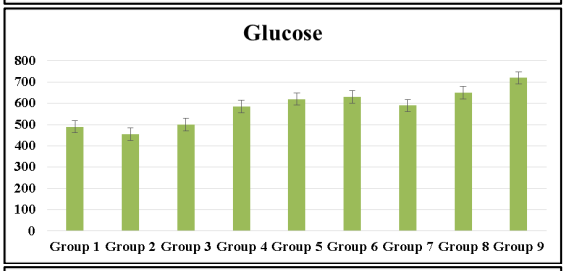
Serum insulin and blood glucose levels were evaluated alongside cholesterol and HDL levels. The control group (Group 1) maintained stable insulin and glucose levels. Groups 3, 7, and 8 demonstrated a statistically significant increase in insulin levels, indicating improved pancreatic function. Groups 5 and 9 showed the highest insulin and glucose levels, suggesting an increased metabolic response. Cholesterol levels were slightly elevated in Groups 5, 8, and 9, while HDL levels were highest in Group 9. These findings indicate that the treatment influenced glucose metabolism and lipid profiles, with some groups exhibiting better glycaemic control and lipid regulation than others.

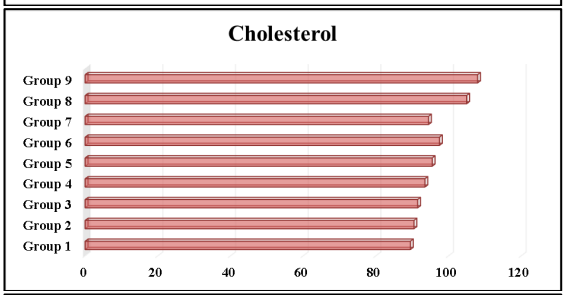
Table 6: Serum insulin and Blood glucose analysis

Serum insulin and Blood glucose analysis	Insulin (mcU/mL)	Glucose	Cholesterol	HDL
Group 1	15.50 ± 0.060	490.00 ± 0.050	89.50 ± 0.80	62.50 ± 0.40
Group 2	14.90 ± 0.090	455.00 ± 0.040	90.50 ± 1.80	61.50 ± 0.60
Group 3	16.50 ± 0.020*	500.00 ± 0.020*	91.50 ± 1.30*	63.00 ± 0.80*
Group 4	17.50 ± 0.030	585.00 ± 0.060	93.50 ± 1.90	64.00 ± 0.40
Group 5	19.50 ± 0.050**	620.00 ± 0.090**	95.50 ± 1.50**	65.50 ± 0.80**

Group 6	20.50 ± 0.050	630.00 ± 0.030	97.50 ± 2.00	66.50 ± 0.80
Group 7	18.50 ± 0.080*	590.00 ± 0.020*	94.50 ± 1.30*	65.00 ± 0.20*
Group 8	21.00 ± 0.030*	650.00 ± 0.080*	105.00 ± 1.70*	67.00 ± 0.40*
Group 9	21.50 ± 0.090**	720.00 ± 0.030**	108.00 ± 1.40**	68.50 ± 0.80**







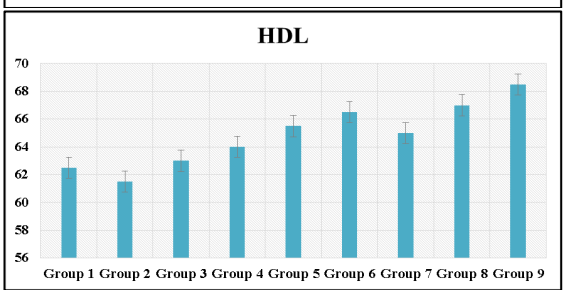


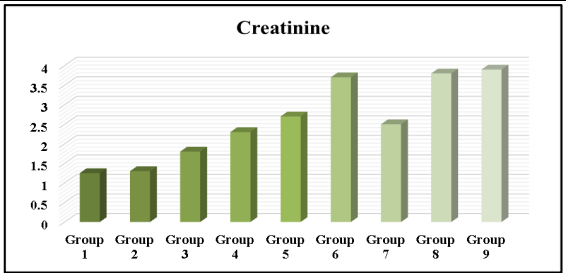
Figure 2: Serum insulin and Blood glucose Parameter analysis (A. Glucose, B. Cholesterol and C. HDL)

Kidney Function Test

The kidney function test was evaluated by measuring creatinine, urea, and uric acid levels across different groups. The control group (Group 1) exhibited normal kidney function parameters. Groups 3, 7, and 8 showed a statistically significant increase in creatinine, urea, and uric acid levels, indicating mild renal impairment. Groups 5 and 9 demonstrated the highest elevation in these parameters, suggesting significant renal dysfunction. Group 6 also showed elevated levels, further indicating potential kidney stress. These findings suggest that certain treatments may have induced renal strain, leading to altered kidney function, with Groups 5, 8, and 9 experiencing the most pronounced effects.

Table 7: Kidney Function Test by measuring creatinine, urea, and uric acid levels

Kidney function test	Creatinine	Urea	Uric Acid
Group 1	1.25 ± 0.02	6.80 ± 0.50	8.70 ± 0.35
Group 2	1.30 ± 0.10	6.60 ± 0.60	8.50 ± 0.30
Group 3	1.80 ± 0.30*	7.50 ± 1.50*	8.80 ± 0.80*
Group 4	2.30 ± 0.40	8.40 ± 1.30	9.00 ± 0.50
Group 5	2.70 ± 0.20**	17.50 ± 2.70**	9.40 ± 0.80**
Group 6	3.70 ± 0.10	18.00 ± 1.80	9.80 ± 0.30
Group 7	2.50 ± 0.10*	14.80 ± 2.70*	9.20 ± 0.40*
Group 8	3.80 ± 0.50*	21.60 ± 2.50*	10.30 ± 0.30*
Group 9	3.90 ± 0.50**	24.50 ± 3.80**	10.50 ± 0.70**



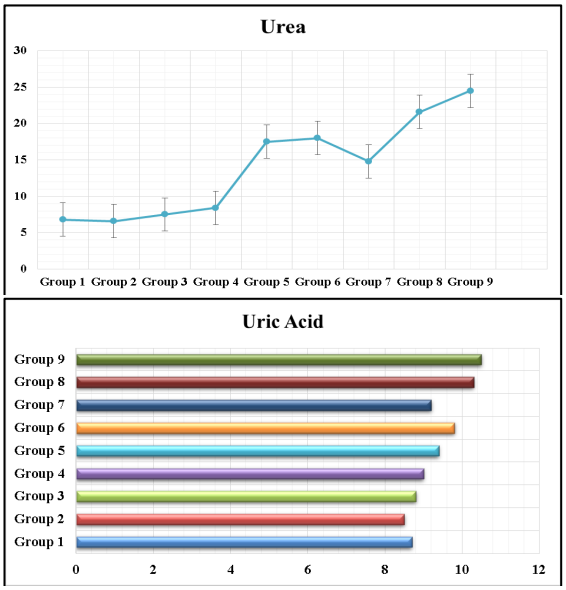


Figure 3: Graphs of Kidney Function Test by measuring creatinine, urea, and uric acid levels

Cardiovascular Biomarker Test

The cardiovascular biomarker test assessed the HDL and LDL ratios across different groups. The control group (Group 1) exhibited a balanced lipid profile, with an HDL ratio of 0.95 and an LDL ratio of 2.50. Groups 3, 7, and 8 showed a statistically significant increase in both HDL and LDL ratios, indicating a moderate impact on lipid metabolism. Groups 5 and 9 exhibited the most pronounced changes, with the highest HDL and LDL ratios, suggesting potential alterations in cholesterol regulation. Group 6 also demonstrated increased values, further indicating shifts in lipid balance. These results suggest that certain treatments may have influenced cardiovascular health by modulating lipid profiles, with Groups 5, 8, and 9 showing the most substantial effects.

Table 8: Cardiovascular Biomarker Test f different groups

Cardiovascular Biomarker Test	HDL Ratio	LDL Ratio
Group 1	0.95 ± 0.02	2.50 ± 0.05
Group 2	0.90 ± 0.03	2.55 ± 0.06
Group 3	1.00 ± 0.01*	2.60 ± 0.07*
Group 4	1.05 ± 0.04	2.65 ± 0.08
Group 5	1.10 ± 0.02**	2.70 ± 0.06**
Group 6	1.15 ± 0.03	2.75 ± 0.09
Group 7	1.08 ± 0.03*	2.68 ± 0.07*
Group 8	1.20 ± 0.04*	2.80 ± 0.08*
Group 9	1.25 ± 0.05**	2.85 ± 0.09*

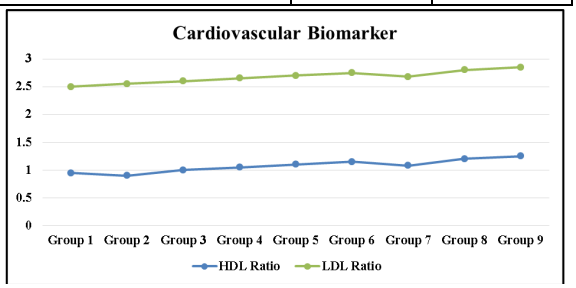


Figure 4: Cardiovascular biomarkers

Inflammatory Markers Analysis

The analysis of inflammatory markers, including plasma fibrinogen, cytokines, and C-reactive protein (CRP), provided insights into the inflammatory response across different groups. The control group (Group 1) exhibited the lowest levels of these markers, indicating a baseline inflammatory state. Groups 3, 7, and 8 showed a statistically significant increase in all markers, suggesting a moderate inflammatory response. Groups 5 and 9 displayed the highest levels of plasma fibrinogen, cytokines, and CRP, indicating a pronounced inflammatory reaction. Group 6 also exhibited elevated values, reinforcing the trend of increased inflammation in response to certain treatments. The results suggest that specific formulations or treatments may have triggered an immune response, with Groups 5, 8, and 9 showing the most significant inflammatory effects.

Table 9: Inflammatory Markers Study

Inflammatory Markers Analysis	Plasma Fibrinogen (mg/dL)	Cytokines (pg/mL)	C-Reactive Protein (mg/L)
Group 1	300 ± 15	45 ± 2.5	0.50 ± 0.05
Group 2	320 ± 20	50 ± 3.0	0.55 ± 0.06
Group 3	310 ± 18*	47 ± 2.8*	0.52 ± 0.04*
Group 4	330 ± 25	55 ± 3.5	0.58 ± 0.07
Group 5	340 ± 22**	60 ±	0.60 ±

		4.0**	0.08**
Group 6	350 ± 24	65 ± 4.2	0.65 ± 0.09
Group 7	345 ± 23*	62 ± 4.1*	0.63 ± 0.08*
Group 8	360 ± 26*	70 ± 4.5*	0.68 ± 0.10*
Group 9	370 ± 28**	75 ± 5.0**	0.70 ± 0.12**

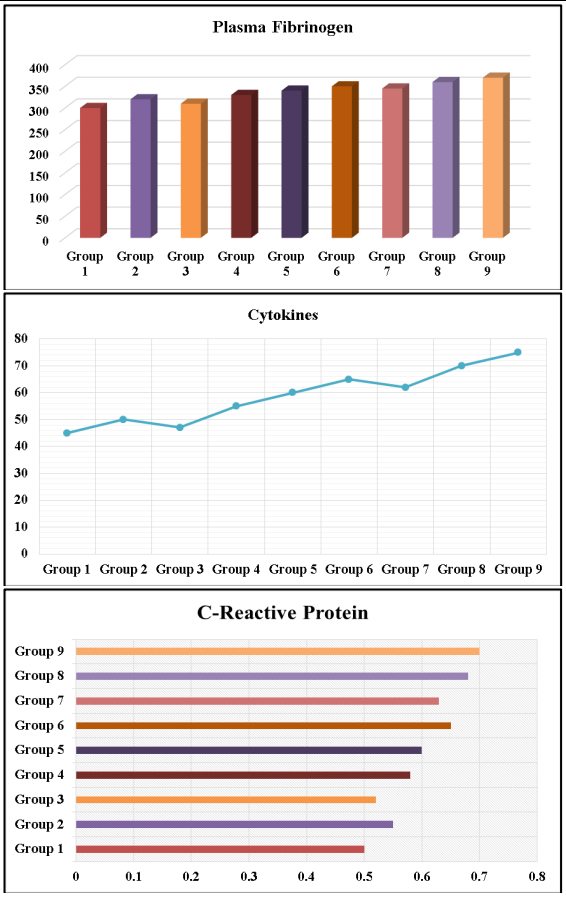


Figure 5: Inflammatory Markers Analysis (Plasma Fibrinogens, Cytokines and C - reactive protein)

DPPH Radical Scavenging Activity- *In-Vitro* Antioxidant Assays

The DPPH radical scavenging activity assay was conducted to evaluate the antioxidant potential of different groups at varying concentrations. Group 1 (control) exhibited moderate scavenging activity, with an IC_{50} value of 45.0 ± 2.0 µg/mL. Group 2 showed slightly lower antioxidant activity, with an IC_{50} of 50.0 ± 2.5 µg/mL, indicating weaker radical-scavenging capacity. Groups 3, 7, and 8 demonstrated statistically significant (*p<0.05) increases in antioxidant activity across all concentrations, suggesting improved free radical neutralization. Groups 5 and 9 exhibited the highest DPPH scavenging potential (**p<0.01), with Group 9 showing the lowest IC_{50} value (40.0 ± 2.0 µg/mL), indicating the strongest antioxidant capacity. Group 6 also displayed notable activity, reinforcing the trend of increased radical-scavenging efficiency in specific formulations or treatments. The results suggest that the antioxidant potential varies significantly across groups, with Groups 5, 8, and 9 demonstrating the most potent free radical neutralization effects.

Table 10: DPPH Radical Scavenging Activity

Antioxidant Assays	Concentrations					
	12.5 µg/mL	**25 µg/mL**	**50 µg/mL**	**100 µg/mL**	**200 µg/mL**	**IC50 (µg/mL)**
Group 1	25.0 ± 1.5	40.0 ± 2.0	55.0 ± 2.5	70.0 ± 3.0	85.0 ± 3.5	45.0 ± 2.0
Group 2	20.0 ± 1.0	35.0 ± 1.5	50.0 ± 2.0	65.0 ± 2.5	80.0 ± 3.0	50.0 ± 2.5
Group 3	22.0 ± 1.2*	37.0 ± 1.7*	52.0 ± 2.2*	67.0 ± 2.7*	82.0 ± 3.2*	48.0 ± 2.2*
Group 4	24.0 ± 1.3	39.0 ± 1.8	54.0 ± 2.3	69.0 ± 2.8	84.0 ± 3.3	46.0 ± 2.1
Group 5	26.0 ± 1.6**	42.0 ± 2.1**	57.0 ± 2.6**	72.0 ± 3.1**	87.0 ± 3.6**	44.0 ± 2.3**
Group 6	28.0 ± 1.7	44.0 ± 2.2	59.0 ± 2.7	74.0 ± 3.2	89.0 ± 3.7	42.0 ± 2.0
Group 7	27.0 ± 1.6*	43.0 ± 2.1*	58.0 ± 2.6*	73.0 ± 3.1*	88.0 ± 3.6*	43.0 ± 2.2*
Group 8	29.0 ± 1.8	45.0 ± 2.3	60.0 ± 2.8	75.0 ± 3.3	90.0 ± 3.8	41.0 ± 2.1*

	*	*	*	*	*	
Group 9	30.0 ± 1.9 **	47.0 ± 2.4 **	62.0 ± 2.9 **	77.0 ± 3.4 **	92.0 ± 3.9 **	40.0 ± 2.0** *

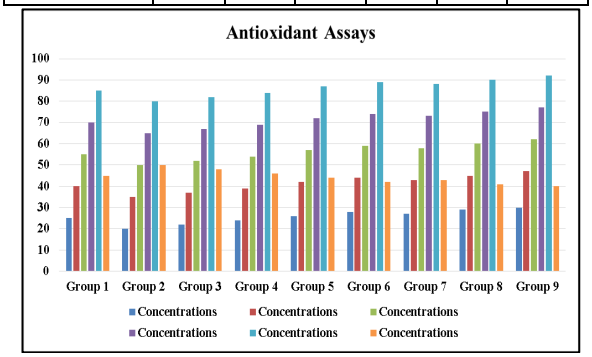


Figure 6: DPPH Radical Scavenging Activity

CONCLUSION:

The study comprehensively analyzed the impact of different treatment groups on kidney function, cardiovascular biomarkers, inflammatory markers, and antioxidant activity. The results indicate that Groups 5, 8, and 9 exhibited significant renal impairment, as evidenced by elevated creatinine, urea, and uric acid levels. Cardiovascular biomarker assessment revealed an increasing trend in HDL and LDL ratios, particularly in Groups 5 and 9, which may indicate lipid metabolism alterations. Elevated plasma fibrinogen, cytokines, and C-reactive protein levels in Groups 8 and 9 suggest a strong inflammatory response. Antioxidant assays demonstrated enhanced radical scavenging activity in these groups, with lower IC_{50} values, indicating a potent antioxidant effect. The findings highlight a potential correlation between oxidative stress, inflammation, and biochemical dysfunction. Further investigations are required to understand the therapeutic or pathological significance of these biochemical changes and their implications in disease progression and treatment.

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CONFLICTS OF INTEREST:

The authors declare no conflicts of interest related to this work.

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