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The Ameliorative Potentials of Naringin Against Sodium Cyanide Toxicity in Male Wistar Albino Rats (*Rattus norvegicus*)

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Abstract

Environmental pollution, particularly from industrial activities, has led to the widespread release of toxic substances like cyanide, disrupting ecosystems and human health. By virtue of inhibiting cytochrome c oxidase, Cyanide disrupts cellular respiration and induces oxidative damage. This study examines the protective effects of naringin, a flavonoid from citrus fruits, against sodium cyanide (NaCN)-induced toxicity in male Wistar rats. 10 months old, 24 rats were grouped into control, NaCN-treated (1.2 mg/kg), and NaCN (1.2 mg/kg) with low (50 mg/kg) or high (100 mg/kg) doses of naringin for 30 days. NaCN exposure caused anaemia, thrombocytopenia, and elevated White Blood Cell (WBC) count, suggesting inflammatory responses. Naringin co-treatment improved these parameters dose-dependently. Elevated levels of liver and kidney markers, such as aspartate aminotransferase, alanine aminotransferase, alkaline phosphatase, creatinine, and urea due to NaCN were reduced with naringin, supported by histological evidence of tissue recovery, including reduced hepatocyte degeneration and glomerular atrophy, confirming hepatoprotective and nephroprotective effects. Pancreatic beta-cell function and islet structure also improved with naringin. NaCN-induced reproductive toxicity, shown by decreased follicle-stimulating hormone, luteinising hormone, testosterone levels, sperm count ($p<0.05$) and testicular damage, was alleviated significantly, particularly with higher doses, promoting spermatogenesis. Notably, it promoted regeneration of spermatogonia, enhanced germ cell production, and increased sperm count in the testes, reversing NaCN-induced reproductive toxicity. Naringin enhanced the antioxidant enzyme activity of superoxide dismutase, catalase, glutathione-s-transferase, glutathione peroxidase, and reduced lipid peroxidation, demonstrating its ability to counter oxidative stress. These results highlight naringin's role in mitigating NaCN toxicity, repairing tissue damage, and restoring physiological and reproductive health, making it a promising candidate for managing oxidative stress-related conditions.

Major Findings: Naringin significantly ameliorated sodium cyanide-induced toxicity in male Wistar rats by restoring hematological, biochemical, hormonal, and antioxidant parameters towards normal range. The high-dose naringin effectively reversed hepatotoxicity, nephrotoxicity, pancreatic damage, and testicular degeneration by improving tissue architecture including sperm count. The enhanced antioxidant enzyme activity, reduced lipid peroxidation will demonstrate strong protective effects against oxidative stress. These findings establish naringin as a promising therapeutic agent for monitoring cyanide toxicity.

Keywords: Antioxidant, Flavonoid, Hepato-Renal, Naringin, Sodium Cyanide

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1. Introduction

Chemicals have become essential components of human existence, serving a crucial function in supporting various industrial operations, enhancing agricultural output, advancements the management and mitigation of numerous illnesses. Cyanides are both natural and anthropogenic. It is used to regulate animal populations[1]. As a result of its remarkable efficiency in causing rapid toxic effects, this substance has found application as a xenobiotic weapon in chemical warfare, with historical evidence linking back to the times of Nero and prehistoric Rome[2]. Its extensive usage in industrial settings encompasses electroplating, plastic fabrication, chemical synthesis, and mining practices like pulverising premium ores and heap-leaching inferior ores worldwide. A wide array of natural sources contain cyanide, encompassing diverse beans, leaves, fruits, and roots, *e.g.*, sorghum, bamboo, cassava, *etc*[3]. Multiple epidemiological studies conducted in populations that consume cassava have confirmed a correlation between exposure to cyanide and the occurrence of spastic paraparesis, amblyopia, ataxia, or tropical ataxia[4].

Cyanide exposure, which poses risks to both humans and other organisms, can arise from tobacco smoke and industrial emissions containing free cyanide. Additionally, certain drugs, including amygdalin and sodium nitroprusside, have the potential to release cyanide ions during metabolism[5]. Cyanide, with a molecular weight of 65.12, is a compact molecule capable of efficiently traversing membranes and engaging in direct interactions with cellular metabolism[6]. In instances of fatal exposure, cyanide can impede the functioning of the COX enzyme system, leading to swift demise as a result of respiratory and Central Nervous System (CNS) failure. The potential hazards connected to prolonged or repeated proximity to cyanide in various industries are extensive and pervasive.

The cyanide ion (CN-) plays a crucial role as the 'active agent' in both the gold leaching process and its impact on the toxicity of biological systems. Numerous chemical species contain cyanide, each exhibiting distinct levels of toxicity. Cyanide can either exist as a free ion or combine with a wide range of elements[7]. Potassium Cyanide (KCN), Sodium Cyanide (NaCN), and Hydrocyanic Acid (HCN) are the most commonly encountered forms of cyanide. In the United States, cyanides have been found in the soil beneath hazardous waste locations and surface water[8]. Free cyanide, regardless of its source, is recognised as the primary toxic agent and the only biologically meaningful expression of cyanide toxicity[9]. The precise procedure by which cyanide exerts its implications is not yet completely understood. It is established that cyanide inhibits mitochondrial respiration, implementing the production of superoxide anion.

Cyanide exerts its fast-acting, poisonous effects by binding to key iron-containing enzymes that are essential for cellular oxygen utilisation. Consequently, tissues are rendered incapable of effectively utilising oxygen from the bloodstream. The blockage of the enzyme COX in the mitochondrial respiratory chain leads to toxicity, disrupting the oxygen-dependent production of energy molecules and causing the cells to switch to oxygen-independent metabolic pathways[10]. Nevertheless, by utilising an additional mitochondrial enzyme called rhodanase, smaller quantities of cyanide can be effectively eliminated from the body without inducing any adverse effects. Rhodanase plays a critical part in converting the majority of ingested cyanide into a less toxic metabolite known as thiocyanate (SCN^-). Rapid exposure to cyanide results in a quick suppression of COX activity, leading to a lack of energy in the affected tissue[11]. The brain and spinal cord (CNS) are thought to be the main sites where cyanide exerts its effects.

Numerous cellular defence pathways have been established by organisms to effectively regulate the quantity of Reactive Oxygen Species (ROS) and mitigate the adverse effects caused by free radicals. These defence mechanisms operate optimally under normal metabolic conditions, ensuring the maintenance of ROS levels within a safe range. The cyanogenic glycosides that can be found in plants like cassava roots have the ability to release cyanide as they undergo metabolism in the intestines[12]. Multiple investigations have shown that acute cyanide toxicity in humans can be attributed to suicide attempts or accidental poisoning through the ingestion or inhalation of cyanide. In most instances, exposure to large amounts of acute cyanide results in the mortality of the individual. The fatality is believed to be a consequence of the depression of the CNS and the suppression of cerebral COX enzyme function[13].

Within the human body, the detoxification of cyanide primarily occurs through enzymatic conversion to a significantly less harmful substance called SCN. This conversion process is mainly facilitated by dietary amino acids that contain sulphur, such as cysteine and methionine[14]. Sodium nitrite and sodium hyposulfite are

commonly used together as the primary treatment for cyanide poisoning[15]. Different C=O containing groups, including sodium pyruvate and 2-oxoglutarate, have been shown to counteract cyanide toxicity by forming hydroxy-nitrile intermediate[16]. Cyanide has been extensively studied as it has been found to hinder the function of numerous enzymes, including those that serve as antioxidants. Moreover, exposure to cyanide has been shown to interrupt crucial intracellular processes, setting off a series of toxic events. Among these effects, oxidative stress, a harmful consequence consistently associated with cyanide toxicity, has been frequently highlighted. Research has shown that cyanide exposure may cause the formation of ROS in the rat brain and result in oxidative degradation of lipids[17]. Bioflavonoids are recognised for their strong bioactive properties in combating free radicals and oxidative stress[18]. According to Van Acker *et al*.,[19] the presence of one or more aromatic -OH groups in the chemical structure of flavonoids is attributed to their radical scavenging activity.

Naringin, a flavonoid glycoside abundantly found in citrus fruits, has garnered significant attention for its multifaceted pharmacological properties. It has been documented to exhibit strong antioxidant capabilities by scavenging free radicals and enhancing the activities of endogenous antioxidant enzymes, thereby mitigating oxidative stress-induced damage[18,20]. Furthermore, naringin has demonstrated notable anti-inflammatory activity by suppressing the expression of pro-inflammatory cytokines, such as tumour necrosis factor-α (TNF-α), interleukin-6 (IL-6), and interleukin-1β (IL-1β), and modulating the necrosis factor – κB (NF-κB) signalling pathway[21]. These properties make it an excellent candidate for countering inflammation-driven cellular damage. Additionally, naringin is recognised for its hepatoprotective effects, as it can prevent liver damage induced by toxins such as carbon tetrachloride and ethanol by preserving liver architecture and reducing elevated liver enzymes[22]. It has also been shown to enhance mitochondrial function and reduce lipid peroxidation, further contributing to its protective effects on vital organs[23]. Naringin's role in cardiovascular health has been extensively studied, with evidence suggesting its ability to lower serum cholesterol and triglyceride levels, improve endothelial function, and reduce atherogenesis through lipid metabolism modulation[24]. In the context of neuroprotection, naringin has been reported to protect neuronal cells from oxidative and excitotoxic damage by regulating apoptosis-related proteins and promoting neurotrophic factors[25]. These neuroprotective properties highlight its therapeutic potential in managing neurodegenerative conditions. Its ability to inhibit Advanced Glycation End Product (AGEs) formation and counter hyperglycemia-related oxidative damage has positioned naringin as a promising agent for diabetes management[26]. Moreover, naringin's metal-chelating properties have been explored in studies focusing on its efficacy against heavy metal toxicity, where it reduces metal ion-induced oxidative damage by binding to free metal ions. The compound also demonstrates promising anti-cancer properties, as it induces apoptosis in various cancer cell lines by targeting pathways like phosphoinositide 3-kinase/protein kinase B (PI3K/Akt) and mitogen-activated protein kinase/extracellular signal-regulated kinase (MAPK/ERK)[27].

Naringin, a polyphenolic citrin compound, undergoes conversion into naringenin, a more absorbable form, with the help of L-rhamnosidase A and β-D-glucosidase enzymes. As earlier mentioned, naringin showcases diverse medicinal and therapeutic characteristics like beta-oxidation of lipids[28], anti-apoptotic[29], anti-diabetic[30], neuroprotective[31], anti-inflammatory[32], wound-healing[33], metal-chelating, microbicidal, anti-mutagenic, carcino-preventive[34], free radical-trapping, and oxidation inhibitor properties. The capability of naringenin to regulate cytochrome P450-dependent monooxygenase, which is the key agent responsible for metabolising various foreign substances. Furthermore, Van Acker *et al*.[19] found that naringenin, the non-sugar moiety of naringin, can act as a chain-alleviating free-radical scavenger in hepatic membrane-bound microsomes, similar to a-tocopherol. To our best understanding, there is currently no existing study investigating the impact of naringin on toxicity induced by Sodium cyanide. Consequently, we assessed the defensive impact of naringin against Sodium cyanide toxicity by analysing changes in blood parameters and histology, along with serum indicatory biomarkers, in Wistar albino rats.

2. Methodologies

2.1 Animal Rearing and Maintenance

In the present study, the male Wistar rats (*Rattus norvegicus*) of body weight 200±10 grams were received from the Department of Zoology, Karnatak University, Dharwad, and reared in the animal facility given standard

circumstances. A period of two weeks was provided for the animals to adjust to the conditions before the experiment in a 12-h cycle of daylight and darkness and 28±3°C ambient temperature, humidity 56±4%. Housing of rats was done in polypropylene cages and provided with commercial food pellets (VRK Nutritional Solutions, Sangli, India) and pure water *ad libitum*.

2.2 Chemicals

Sodium cyanide (CAS No. 143-33-9) and Naringin (CAS No. 10236-47-2) were procured from Himedia Laboratories Pvt. Ltd., Mumbai, Maharashtra. The rest of the chemicals, including Haematoxylin, Eosin (water soluble), Benzene, Paraffin wax, and were of analytical grade and purchased from Sisco Research Laboratories Pvt. Ltd.

2.3 Exposure and Workflow

In the current investigation, after 10 days of acclimation, 24 rats were categorised into 4 groups (n=6).

Group 1 (G1): The control group received normal food and water

Group 2 (G2): Dosed with Sodium cyanide (1.2 mg/kg b.w.)

Group 3 (G3): Dosed with Sodium cyanide (1.2 mg/kg b.w.) + Naringin (50 mg/kg b.w.)

Group 4 (G4): Dosed with Sodium cyanide (1.2 mg/kg b.w.) + Naringin (100 mg/kg b.w.)

The dose of sodium cyanide (1.2 mg/kg body weight) was selected based on previously published studies in our laboratory and conforms to 1/5th of the reported LD_{50} value (6.44 mg/kg b.w.) for NaCN in rodents, as described by Shivanoor and David[35]. This sub-lethal dose is in line with OECD toxicity guidelines, which recommend using a fraction of the LD_{50} to model chronic or sub-acute toxicity while minimising mortality, considering the No Observed Adverse Effect Level (NOAEL)[36]. Using the same NaCN dose across groups reduces inter-group variability, improving the statistical validity of the results and allowing for the evaluation of whether naringin's protective effect is dose-dependent. Hence, it serves as a gold standard approach for assessing protective interventions under controlled toxic exposure.

The non-fasted rats were given a dose of 1 mL/100 g b.w. of NaCN orally in the morning, specifically between 9:00 and 10:00 am, for 30 days. After a period of 30 days of exposure to Naringin, the rats underwent overnight fasting. Following a 24-h period, they were decapitated by being subjected to sequential anaesthesia using sodium pentobarbital for a duration of 30 to 40 seconds. The cardiac puncture method was used to draw blood. Upon blood collection, each blood sample was carefully stored in distinct tubes for the analysis of hormones and serum parameters. Subsequently, the liver, kidney, pancreas, and testes were harvested. The samples were separated into two parts: one part was utilised for biochemical analysis, while the other part was prepared for histopathological evaluation.

2.4 Haematology

Blood samples for haematological analysis are collected using EDTA tubes to prevent clotting. After cardiac puncture, blood is drawn into the tube and mixed gently to ensure proper anticoagulation. Key parameters such as erythrocyte (RBC) count, haemoglobin (Hb), Hematocrit (Hct), platelet count, and White Blood Cell (WBC) count are analysed using either a Neubauer chamber or an automated analyser. The RBC count is determined by examining a diluted blood sample under a microscope or using the analyser's optical or impedance methods. Haemoglobin levels are measured using a colorimetric assay, while hematocrit is evaluated by centrifuging the sample or calculated by the analyser. Platelet and WBC counts are similarly obtained either manually or using automated methods. Ensuring accurate results involves quality control measures, such as regular calibration of equipment and the use of control samples[37].

2.5 Hormone Analysis

Cardiac puncture technique was utilised to procure blood samples from the subjects while they were anesthetised with sodium pentobarbital, and the samples were stored in centrifuge tubes. Subsequently, the blood was permitted to form a clot at ambient temperature for 10 min prior to undergoing centrifugation at 500g×g for 5 min at 4°C. The serum obtained was then transferred to separate vials for the evaluation of LH, FSH, and Testosterone concentrations using an entirely computerised bi-directional Integrated chemiluminescent immunoassay.

2.6 Epididymal Sperm Concentration Assessment

The sperm cell concentration in the sample was evaluated using a hemocytometer. 1 mL portion of the sperm

solution was combined with 9 mL of a diluting medium prepared by dissolving 5 g of $NaHCO_3$, 1 mL of 35% formalin, and 25 mg of eosin in 100 mL of distilled water. Following this, 10 µL of the diluted mixture was loaded into each chamber of the hemocytometer and left to settle for 5 min. The concentration of sperm in the epididymal sample was quantified as millions of cells per millilitre of the prepared solution under 40x magnification using a phase-contrast microscope (Olympus CH20i). The final value was calculated using a predefined formula[38].

Sperm count = Total number of sperm in 5 squares5000100(Sperm mL)

2.7 Biochemical Analysis

Serum was obtained by allowing blood samples to clot at room temperature for 30 minutes, followed by centrifugation at 1500 rpm for 10 min at 4°C in a refrigerated centrifuge[39]. The clear serum layer was carefully separated for analysis or stored at -20°C for later use. Biochemical parameters were measured using a UV-visible spectrophotometer (Hitachi-2800) and reagent kits as per the manufacturer's instructions. The bromocresol green colorimetric method was employed to estimate serum albumin, while ALP activity was determined using a kinetic assay with a p-nitrophenyl phosphate substrate[40]. The enzymatic urease method was used to measure BUN, and the CHOD-PAP method was applied for total cholesterol analysis[41]. Creatinine levels were assessed using the Jaffe reaction or an enzymatic method, and HDL cholesterol was analysed using either the precipitation technique or direct enzymatic methods[40]. LDL cholesterol was calculated using the Friedewald formula or determined enzymatically[42]. Enzymatic kinetic methods were used to evaluate SGOT (AST) and SGPT (ALT) activities. Triglycerides were measured by the enzymatic glycerol phosphate oxidase method, total bilirubin by the diazo method, and uric acid by the uricase-peroxidase method[41].

2.8 Assessment of Antioxidant Status

Tissues from the liver, kidney, pancreas, and testis were minced into small pieces, and 10% homogenates were prepared using chilled 0.05 M potassium phosphate buffer at neutral pH. The cell lysates were then spun at 6000 rpm for 15 min at a temperature of 4°C, and the resulting supernatant was used to measure the activities of various enzymes, including Glutathione Peroxidase (GPx), Glutathione Reductase (GR), Glutathione S-Transferase (GST), catalase (CAT), and Superoxide Dismutase (SOD), along with Lipid Peroxidation (LPO). LPO levels were determined following the procedure outlined by Yagi *et al.*[43] and expressed as nmol of TBARS produced per mg of protein. CAT activity was analysed using Luck's method[44] and expressed as µmoles of H_2O_2 broken down per minute per mg of protein. The SOD activity was estimated based on the method of Kakkar *et al.*[45], and GPx activity was measured using the Paglia and Valentine technique[46], expressed as U per mg of protein. GR and GST activities were assessed using the Moron *et al.*[47] method and expressed as moles of NADH oxidised per minute per mg of protein and U per mg of protein, respectively.

2.9 Histological Evaluation

For histoarchitectural evaluation, the testes, liver, kidney, and pancreas were harvested. The tissue specimens were immersed in Bouin's fixative for 24 h, followed by dehydration through a series of alcohol gradients, and then incorporated into paraffin wax. The paraffin sections were cut into 5µm thick using a partially-motorised microtome (LeicaRM 2255) and staining of the sections were staining with haematoxylin and eosin (H&E) for microscopic observation. The specimens were visualised and captured employing a phase shift microscope (Olympus BX51, Tokyo, Japan) equipped with a camera (ProgResC3, Jenoptic-Germany).

2.10 Immunohistochemical Examination of the Pancreas

Immunohistochemical analysis was performed to evaluate the expression of anti-insulin antibodies using the peroxidase-labelled Avidin-Biotin method. Pancreatic tissue sections underwent a series of preparation steps, including de-waxing, hydration with distilled water, and washing in Phosphate-Buffered Saline (PBS) for 15 min. Sections were then blocked with 3% bovine serum albumin and incubated with the primary antibody overnight at 4°C. To identify B-cells, an anti-insulin monoclonal rabbit primary antibody (PathnSitu Biotechnologies, India) was employed. Following incubation, the tissue was treated with 10% hydrogen peroxide, washed in PBS, and stained with Diaminobenzidine (DAB) for visualisation. Hematoxylin was used for counterstaining, and the slides were dehydrated and mounted with a coverslip.

For negative controls, slides were stained with an IgG secondary antibody. Insulin-positive cells exhibited brown cytoplasmic staining, which was observed under a light microscope (Olympus BX1, Tokyo, Japan) at 200X magnification, with images captured using a ProgResC3 camera (Jenoptic, Germany).

2.11 Statistical Evaluation and Data Interpretation

The data are shown as the average ± standard deviation (SD). GraphPad Prism 10.4 was used for statistical analysis. Group differences were analysed using one-way ANOVA, with Tukey's post-hoc test for further comparison. Cohen's d analysis was used for the sperm count parameter. Statistical validity was assessed using pvalues of <0.05, <0.01, <0.001, and <0.0001. Assumptions of normality and variance homogeneity were checked before applying ANOVA.

3. Results

3.1 Haematology

This study depicted a statistically substantial reduction in the haemoglobin, RBC, and platelet levels in the sodium cyanide-treated cohort (G2) compared to those of the control (G1). The naringin-treated groups, *i.e.*, G3 and G4, have shown a significant rise ($p<0.05$) in the haemoglobin, RBC, and platelet levels as compared to those of the G2 and G1 groups, as shown in Table 1**.** A notable rise was observed in the WBC and differential count levels in the sodium cyanide-treated group (G2) compared to those of the control (G1) group. The naringin-treated groups, *i.e.*, G3 and G4, have shown a notable reduction ($p<0.05$) in the WBC and differential counts levels compared to those of the G2 group.

3.2 Hormone Analysis

Figure 1a illustrates follicle-stimulating hormone (FSH) levels (mIU/mL) across different experimental groups. A noteworthy decrease ($p<0.0001$) in FSH quantity was perceived in the sodium cyanide-treated group (G2) in reference to the control (G1). In the group treated with sodium cyanide and low-dose naringin (G3), no notable difference was observed in FSH amounts (ns) against G2, indicating minimal recovery. However, high-dose naringin treatment (G4) led to a marked rise ($p<0.0001$) in FSH levels in comparison with G2 and G3, closely matching the levels observed in the control group.

A substantial decrease ($p<0.0001$) in LH quantities was noted in the sodium cyanide-treated group (G2) against the control (G1), indicating suppression of hormone synthesis. The group treated with sodium cyanide and low-dose naringin (G3) demonstrated a notable rise ($p<0.0001$) in LH against G2, but the values reduced considerably ($p<0.0001$) in comparison with the control. High-dose naringin treatment (G4) led to a substantial improvement ($p<0.0001$) in LH in comparison with G2 and G3, with values surpassing those of the control (G1) as depicted in Figure 1b.

Figure 1c shows the testosterone levels (ng/dL) across the different experimental groups. The testosterone quantities were found to be reduced ($p<0.0001$) in the sodium cyanide-treated group (G2) against the control (G1). In the group treated with sodium cyanide and low-dose naringin (G3), testosterone levels indicated a marked rise ($p<0.0001$) against G2, indicating some recovery. However, the high-dose naringin treatment

Table 1. Influence of NaCN and Naringin on haematological parameters

Groups	G1 (Control)	G2 (NaCN 1.2 mg/kg)	G3 (NaCN 1.2 mg/kg+Naringin 50 mg/kg)	G4 (NaCN 1.2 mg/kg+Naringin 100 mg/kg)
Hb (gram%)	12.32333±0.409	9.051667±0.580^{a}	10.88833±0.622^{ab}	12.61667±0.515^{bc}
RBC (10^6/mm^3)	7.58167±0.295	6.53±0.319^{a}	7.14±0.114^{ab}	7.52±0.227^{b}
Platelets (10^5/mm^3)	5.62166±0.223	4.42±0.324^{a}	5.001667±0.26^{ab}	5.57±0.221^{bc}
WBC (Cells/mm^3)	4428.5±226.57	7880±458.55^{a}	6001±316.28^{ab}	4482.3±275.84^{bc}
Eosinophil (%)	3.365±0.139	5.501±0.208^{a}	4.3633±0.284^{ab}	3.295±0.222^{bc}
Monocyte (%)	6.993±0.179	8.468±0.370^{a}	7.44±0.374^{b}	7.068±0.170^{b}
Lymphocyte (%)	53.425±1.559	64.183±1.800^{a}	58.4617±1.699^{ab}	53.248±1.797^{bc}

Values are shown as mean±SD (n = 6). Superscript letters denote significant differences: ^{a} vs. G1, ^{b} vs. G2, and ^{c} vs. G3 ($p<0.0001$).

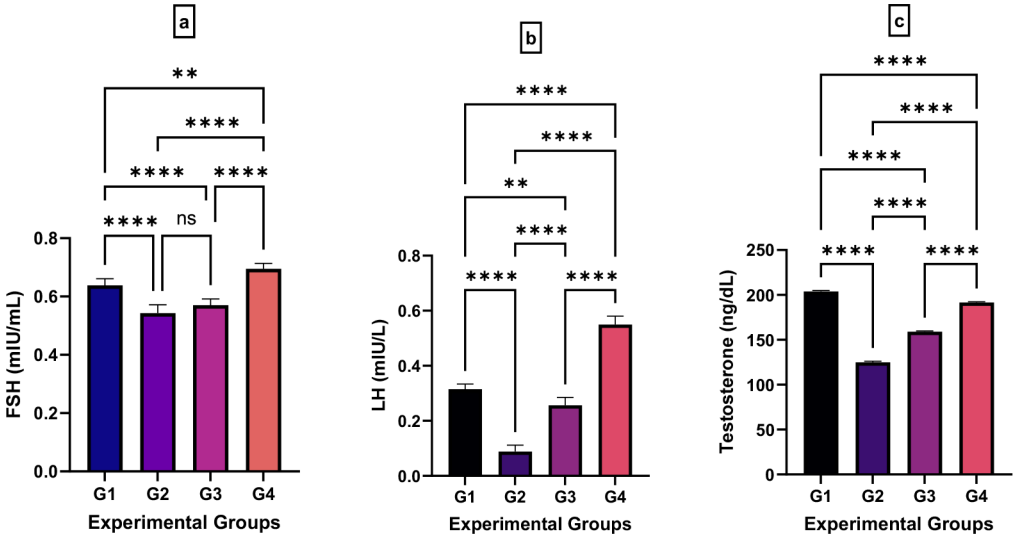


Figure 1. Impact of NaCN and Naringin on Hormone levels. **(A)** Follicle Stimulating Hormone, **(B)** Luteinising Hormone in mIU/L, **(C)** Testosterone in ng/dL.
Data are shown as average ± SD (n = 6). Asterisks signify levels of substantial variation between the experimental groups: **** for p<0.0001, *** for p<0.001, ** for p<0.01, and * for p<0.05.

(G4) represented a marked rise (p<0.0001) in testosterone levels in comparison with both G2 and G3, approaching the levels observed in the control (G1).

3.3 Epididymal Sperm Concentration Assessment

Figure 2 illustrates sperm count (10^6/mL) across different experimental groups analysed through Cohen's d calculation. Group 2 exhibited a marked decrease in sperm count against the control (G1), as reflected by a large effect size (Cohen's d = 30.26). Group 3 showed a partial recovery with a significantly higher sperm count than G2 (Cohen's d = 21.7), though it remained lower than G1 (Cohen's d = 9.1020). In contrast, Group 4 displayed a marked improvement in sperm count, significantly higher than both G2 (Cohen's d = 31.23) and G3 (Cohen's d = 7.7), with values closely approaching those observed in the control group (Cohen's d = 1.55). These results emphasise the varying impact of treatments across the experimental groups.

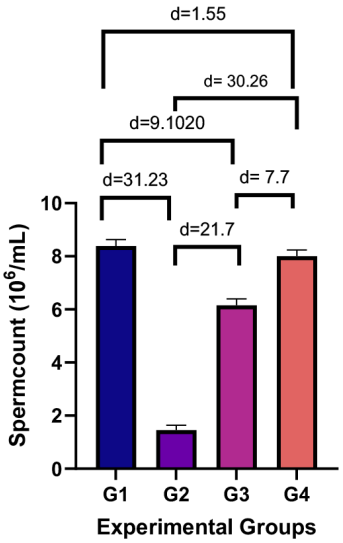


Figure 2. Effect of NaCN and Naringin on Epididymal sperm count.
Values are shown as average ± SD (n = 6). Given Cohen's d values indicate levels of significant effect among the experimental groups: Small: d=0.2 (the difference is noticeable but small), Medium: d=0.5 (a moderate effect), Large: d=0.8 (a substantial effect).

3.4 Serum Biochemistry

Figure 3a-c illustrates the aspartate aminotransferase (AST), alanine Aminotransferase (ALT), and Alkaline Phosphatase (ALP) levels (IU/L), respectively, among different experimental groups. Group 2 showed a substantial elevation (p<0.0001) in AST, ALT, and ALP levels in comparison with the control group (G1). Similarly, Group 3 displayed significantly higher AST, ALT, and ALP levels (p<0.0001) than G1, although slightly lower than G2, suggesting a partial improvement. In contrast, Group 4 demonstrated a notable reduction (p<0.0001) in AST, ALT, and ALP quantities against both G2 and G3, with values nearly

returning to those of the control group. These findings highlight the differential impacts of treatments across the groups.

Figure 4d, e, and f illustrate the levels of total protein (g/dL), creatinine (mg/dL), and blood urea (mg/dL), respectively, across different experimental groups, highlighting the differential effects of treatments. For total protein levels (Graph D), group 2 exhibited a marked rise ($p<0.0001$) against the control group (G1). Groups G3 and G4 also showed higher protein levels than G1, but these variations did not reach statistical significance (ns). No notable variation (ns) was detected between groups 3 and 4. Regarding creatinine levels (Graph E), group 2 demonstrated a marked rise ($p<0.0001$) against G1. Group 3 displayed a partial recovery, with creatinine levels significantly lower than G2 ($p<0.0001$) but still elevated compared to G1 ($p<0.0001$). Group 4 displayed a notable decrease in creatinine levels against G2 ($P < 0.0001$), with no notable variation (ns) in comparison with G3, suggesting substantial improvement. For blood urea levels (Graph F), Group 2 exhibited a marked elevation ($p<0.0001$)

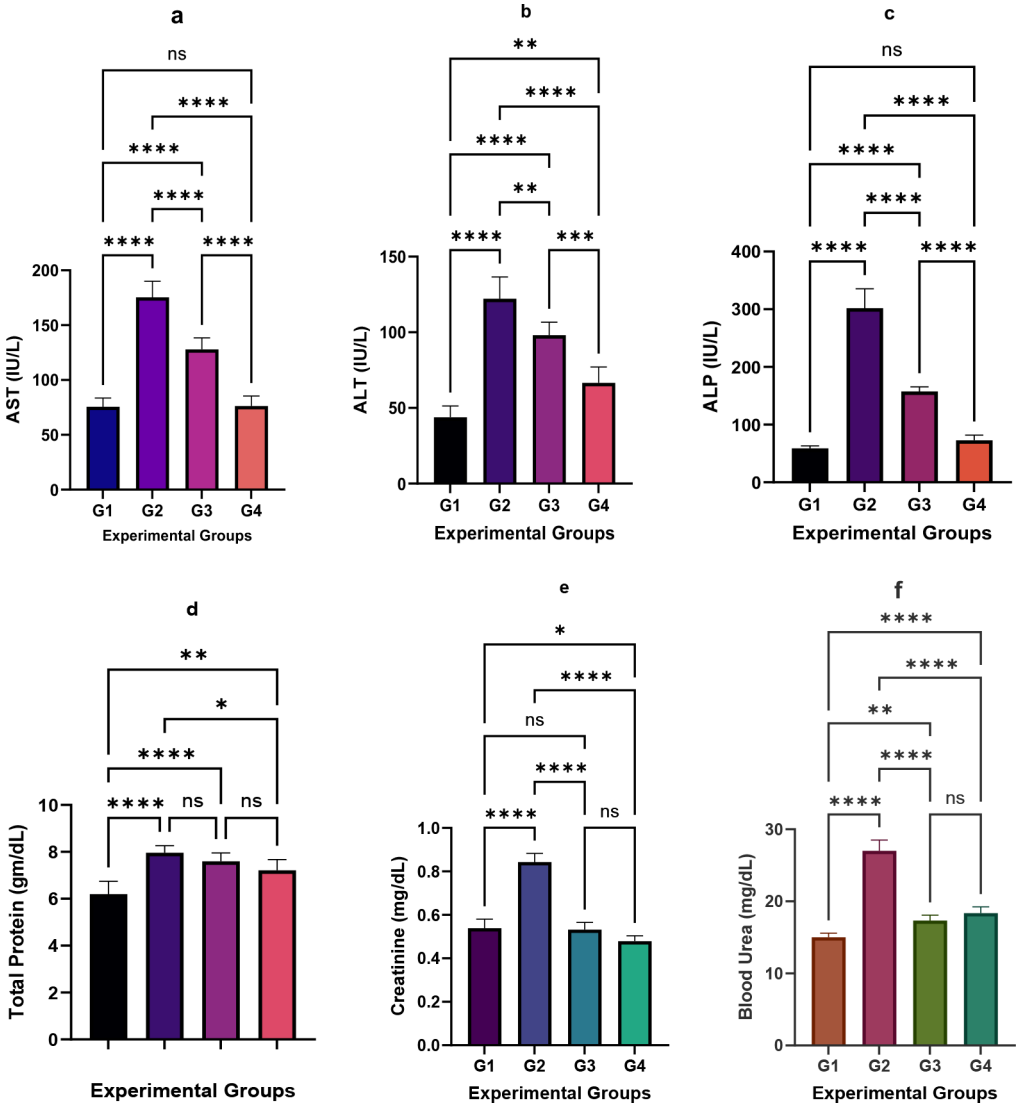


Figure 3. Effect of NaCN and Naringin on Liver and Renal Markers. **A-C** indicates Liver function marker enzymes **(A)** Aspartate aminotransferase (AST), **(B)** Alaline aminotransferase (ALT), **(C)** Alkaline phosphatase (ALP) in IU/L; D-F indicates Kidney function markers, **(D)** Total Protein in gm/dL, **(E)** Creatinine in mg/dL, **(F)** Blood Urea in mg/dL.

Values are shown as average ± SD (n = 6). Asterisks denote the amount of substantial variation among the experimental groups: **** for $p<0.0001$, *** for $p<0.001$, ** for $p<0.01$, and * for $p<0.05$.

against G1. Group 3 showed a substantial reduction in comparison with G2 ($p<0.0001$) but remained elevated relative to G1 ($p<0.01$). Group 4 displayed a notable reduction in blood urea quantities in comparison with G2 ($p<0.0001$), with no notable distinctions (ns) among G3 and G4. In summary, Group 2 consistently showed elevated levels of total protein, creatinine, and blood urea, indicating adverse effects. Groups 3 and 4 demonstrated varying degrees of recovery, with G4 showing the most notable improvement across all three parameters, closely approaching the control group's values.

3.5 Histopathology

Figure 4-7 presents the histological examination of liver, kidney, pancreas, and testis tissues from control, NaCN, and Naringin-treated groups. The liver histology of the control and G4 100 mg/kg b.w. Naringin-treated groups revealed normal hepatocyte arrangements with clear sinusoidal spaces and a central vein, characteristic of a healthy liver where hepatocytes are organised into cords radiating from the central vein and separated by sinusoids containing Kupffer cells (Figure 4A, D). The NaCN-treated group (1.2 mg/kg b.w.) showed significant hepatocyte hypertrophy, central vein distortion, inflammatory infiltration, and

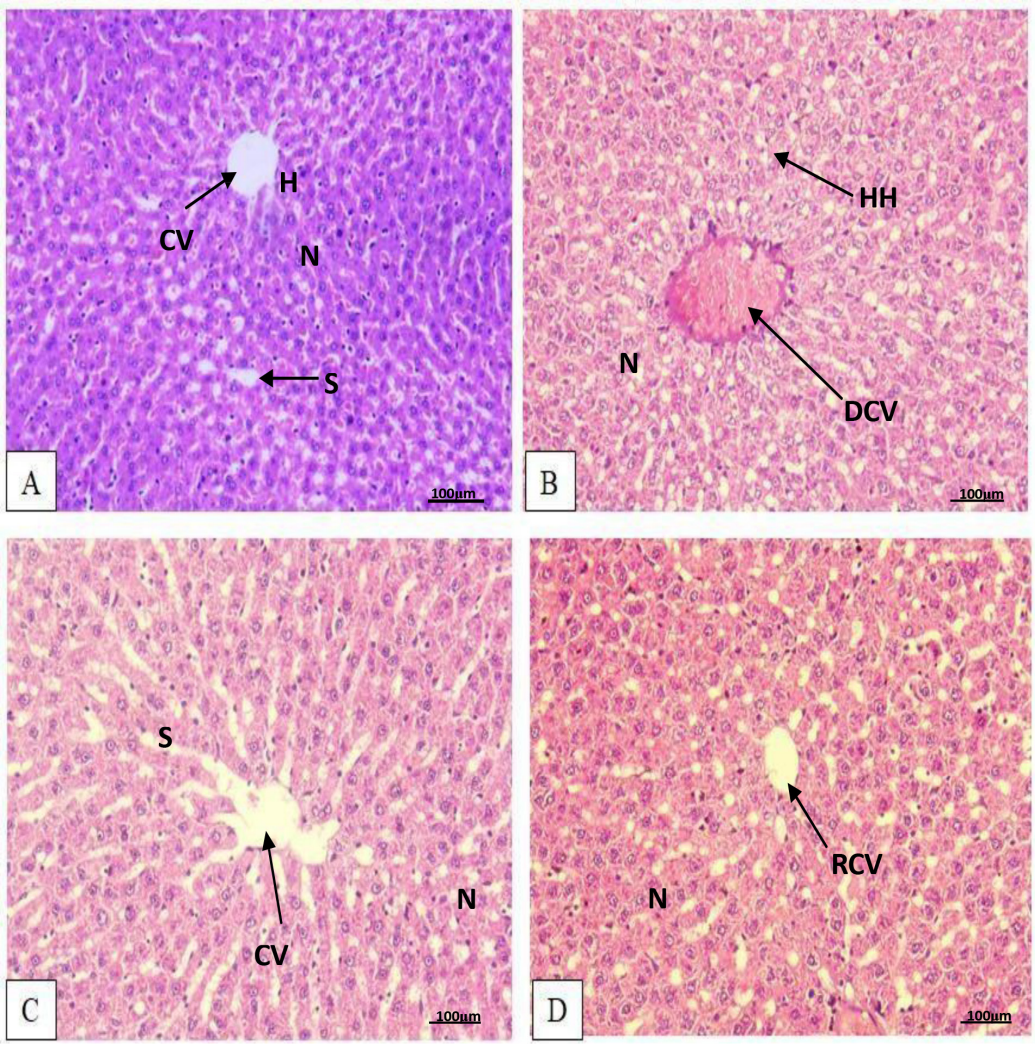


Figure 4. Histoarchitectural changes of Liver **(A-D)** tissue stained with H and E. **(A)** Liver of control rats showing normal artery, central vein, and bile duct, **(B)** Liver of NaCN treated rats showing Hypertrophy of Hepatocytes (HH), Pyknotic nuclei (N), and Distortive/Degenerative central vein (DCV), **(C)** Liver of NaCN+ Naringin 50 mg/kg b.w. treated rats showing Regenerative Central Vein (RCV) with almost normal histoarchitecture of the liver, **(D)** Liver of NaCN+ Naringin 100 mg/kg b.w. treated rats showing RCV, Magnification- 10X, scale- 100 µm, Field of View (FOV)-2.0 mm, Splitting direction- Transverse.

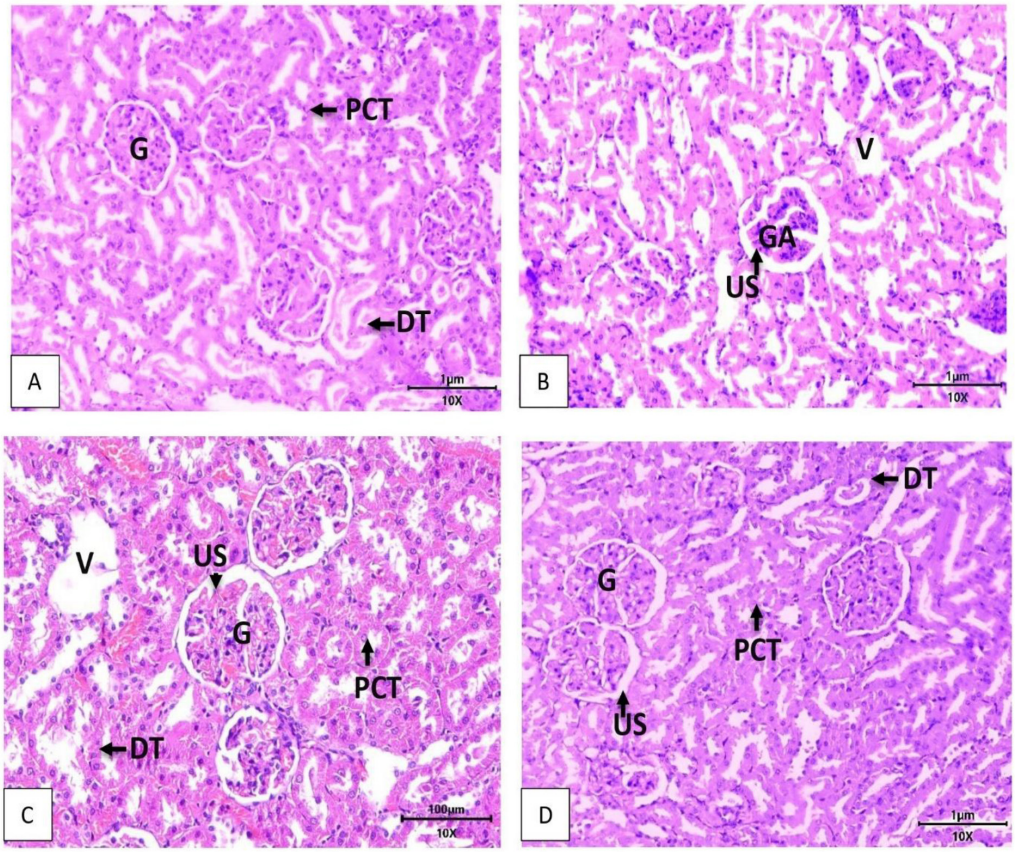


Figure 5. Histoarchitectural changes of Kidney **(A-D)** tissue stained with H and E. **(A)** Kidney of control rats showing normal Glomerulus, PCT, and DCT, **(B)** Kidney of NaCN-treated rats showing Vacuolation (V), dilated Urinary Space (US), and Glomerular Atrophy (GA), **(C)** Liver of NaCN+ Naringin 50 mg/kg b.w. treated rats showing near normal histoarchitecture of the Kidney with US, Glomerulus, PCT, and DCT, **(D)** Liver of NaCN+ Naringin 100 mg/kg b.w. treated rats showing normal histoarchitecture of the Kidney with Glomerulus, PCT, and DCT. Magnification- 10X, scale- 100 µm, Field of View (FOV)-2.0 mm, Splitting direction- Transverse.

localised necrosis (Figure 4B). Conversely, the NaCN + Naringin 50 mg/kg b.w. the treated group exhibited improved liver structure, with regenerated central veins and restored sinusoidal spaces (Figure 4C).

In the kidney, normal histological structures such as intact glomeruli, Proximal Convoluted Tubules (PCT), Distal Convoluted Tubules (DCT), and collecting ducts were observed in the control and G4 100 mg/kg b.w. Naringin-treated groups. Healthy kidneys display compact glomeruli surrounded by Bowman's capsules, PCT with brush borders for reabsorption, and DCT with clearer luminal spaces (Figure 5A, D). The NaCN-treated group (10 mg/kg b.w.) showed glomerular shrinkage, vacuolation in tubular cells, disrupted tubular architecture, and mild interstitial oedema (Figure 5B). The NaCN + Naringin 50 mg/kg b.w. group showed notable recovery, including restored glomeruli and normal tubule morphology (Figure 5C).

In pancreatic sections, control and G4 100 mg/kg b.w. Naringin-treated groups displayed well-preserved histology with structurally normal islets of Langerhans and acinar cells, typical of a healthy pancreas where acinar cells form lobules and islets of Langerhans contain beta, alpha, and delta cells (Figure 6A, D). NaCN exposure at 1.2 mg/kg b.w. led to severe beta cell depletion, disrupted islet architecture, and vacuolation within the pancreatic tissue (Figure 6B). In contrast, the NaCN + Naringin 50 mg/kg b.w. group exhibited partial recovery with signs of beta cell regeneration and improved islet structure (Figure 6C).

Histology of the testis in the control group (Figure 7A) displayed intact seminiferous tubules with properly arranged spermatogenic cells, active spermatogenesis,

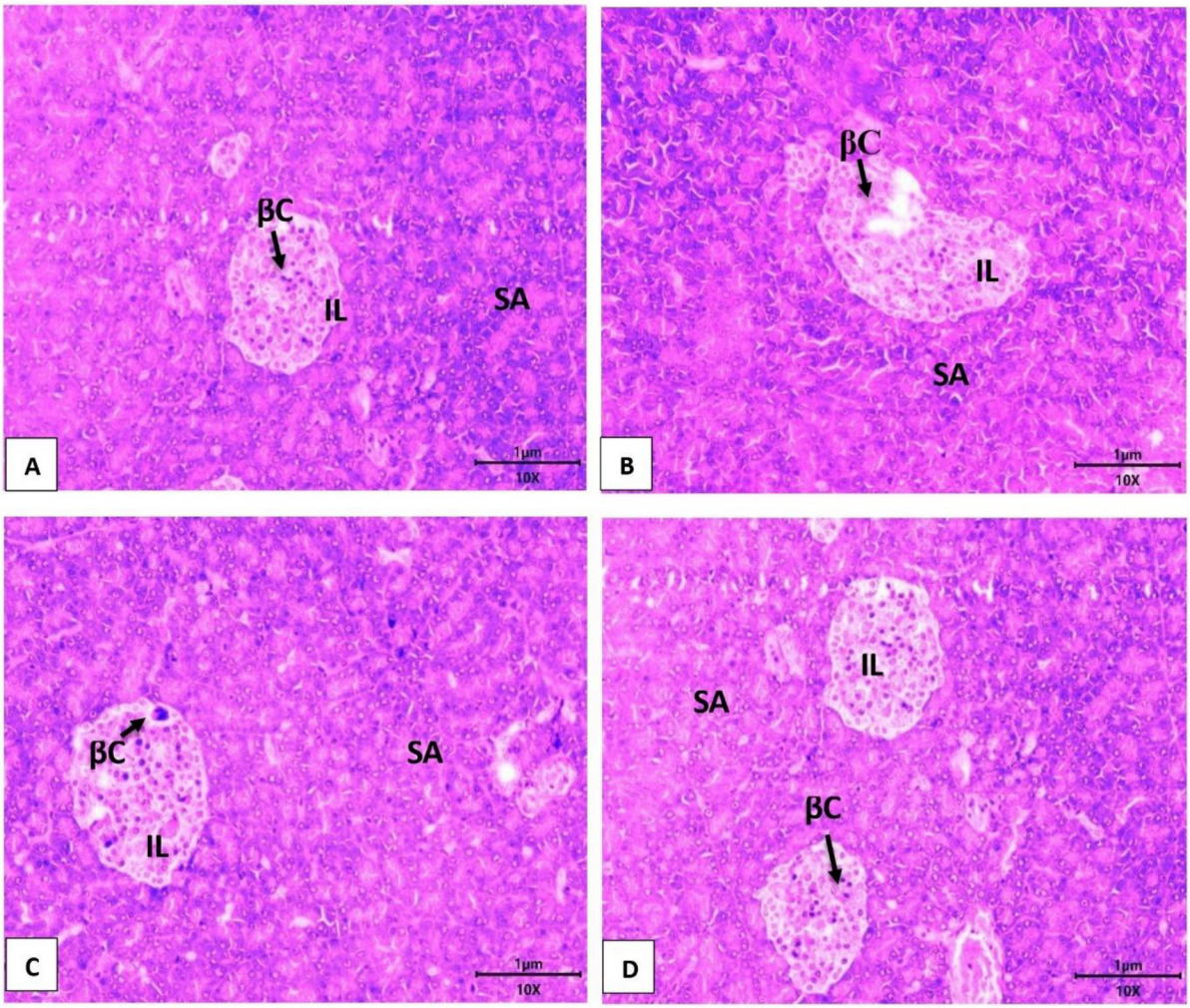


Figure 6. Histopathological changes of Pancreas **(A-D)** tissue stained with H and E. **(A)** Pancreas of control rats showing normal Islets of Langerhans with Beta cells, **(B)** Pancreas of NaCN-treated rats showing Vacuolation (V) and depletion in islets/beta cells number, **(C)** Pancreas of NaCN+ Naringin 50 mg/kg b.w. treated rats showing near normal histoarchitecture of the Pancreas with a moderate number of beta cells, **(D)** Pancreas of NaCN+ Naringin 100 mg/kg b.w. treated rats showing normal histoarchitecture of the Pancreas with Islets of Langerhans and Beta cells. Magnification- 10X, scale- 100 µm, Field of View (FOV)-2.0 mm, Splitting direction- Transverse.

and normal interstitial tissue, as observed in healthy testis, where seminiferous tubules contain spermatogonia, spermatocytes, and mature spermatozoa supported by Sertoli cells and Leydig cells. NaCN treatment (1.2 mg/kg b.w.) resulted in degenerative changes, including atrophied seminiferous tubules, giant spermatogonia, reduced sperm density, and interstitial tissue damage (Figure 7B). The group was treated with NaCN + Naringin 50 mg/kg b.w. showed a partial damage reversal, with improved seminiferous tubules and recovering spermatogenesis (Figure 7C). In the NaCN + Naringin 100 mg/kg b.w. group, testis sections showed nearly normal seminiferous tubule architecture and restored spermatogonia and sperm development (Figure 7D).

3.6 Immunohistochemistry

In the present study, the insulin localisation, representing insulin content in the islet of Langerhans, was done by immunohistochemistry of pancreatic tissue sections of the different groups. The insulin antigen-antibody interaction is indicated by a positive reaction, characterised by the presence of cytoplasmic granules and the appearance of a brown colouration. In Figure 8A Control (G1) group showed a normal distribution of anti-insulin positive

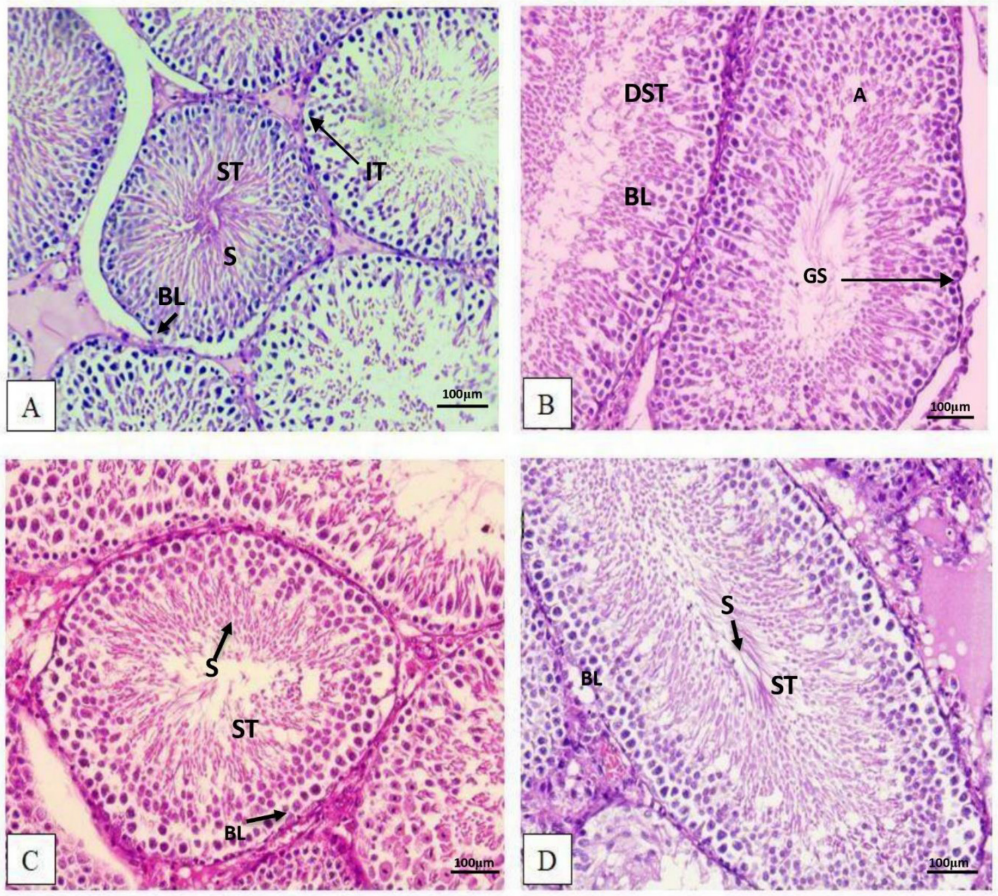


Figure 7. Histoarchitectural changes of testis **(A-D)** tissue stained with H and E. **(A)** Testis of control rats showing normal Seminiferous tubule (ST), Sperm (S), and Interstitial tissue (IT), **(B)** Testis of NaCN treated rats showing Degenerated Seminiferous Tubule, Atrophy (A), and Giant Spermatogonia (GS), **(C)** Testis of NaCN+ Naringin 50 mg/kg b.w. treated rats showing Seminiferous tubules with almost normal histoarchitecture of the testis, **(D)** Testis of NaCN+ Naringin 100 mg/kg b.w. treated rats showing Regenerated seminiferous tubules along with normal spermatogonia and sperm. Magnification- 10X, scale- 100 µm, Field of View (FOV)-2.0 mm, Splitting direction- Transverse.

immune-staining all over the pancreatic islets. The sodium cyanide (G2) group illustrated a reduction in the insulin content of beta cells, as shown in Figure 8B. On the other hand, the naringin-treated groups, *i.e.*, G3 and G4 groups (Figures 8C, D), rats showed a significant elevation in the insulin content compared to that of the sodium cyanide.

3.7 Influence of NaCN and Naringin on Activities of Enzymes with Antioxidant Properties and Lipid Peroxidation in (A) Liver, (B) Kidney, (C) Pancreas, and (D) Testis

The study revealed a marked reduction ($p<0.0001$) in Superoxide Dismutase (SOD) activity across all examined tissues (liver, kidney, pancreas, and testis) in the sodium cyanide-treated group (G2) against control (G1) as depicted in Table 2. In the group receiving sodium cyanide along with low-dose naringin (G3), SOD activity showed a partial recovery ($p<0.0001$) compared to Group 2 but remained substantially lower than that of the control. On the other hand, the high-dose naringin-treated group (Group 4) displayed a notable improvement ($p<0.0001$) in SOD activity relative to Groups 2 and 3, which is nearer to the levels observed in the control group.

The analysis displayed a notable decline ($p<0.0001$) in catalase activity across all evaluated tissues (liver, kidney, pancreas, and testis) in the sodium cyanide-treated group (Group 2) in comparison with the control group (Group 1) as depicted in Table 3. In the group treated with sodium cyanide

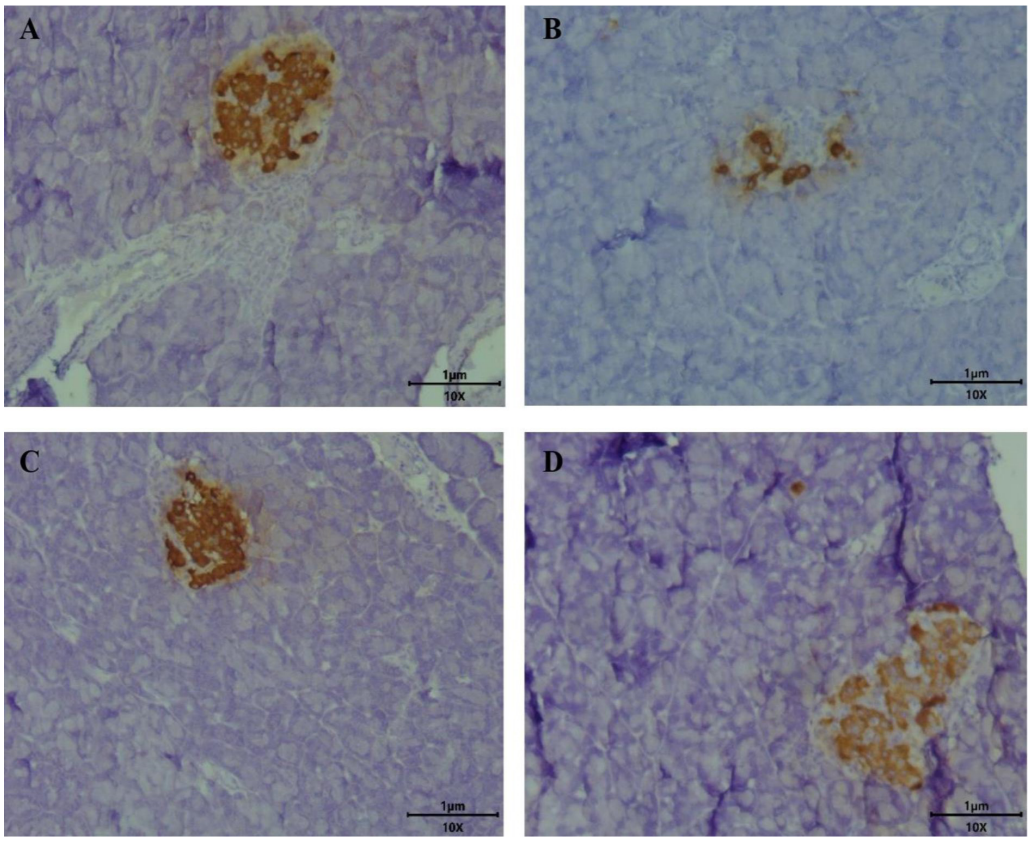


Figure 8. Immunohistochemical staining of the pancreas. **(A)** Pancreas of control rats showing normal Islets of Langerhans with Beta cells, **(B)** The pancreas of NaCN-treated rats shows reduced insulin content, **(C)** Pancreas of NaCN+ Naringin 50 mg/kg b.w. treated rats showing the Pancreas with a moderate amount of insulin expression, **(D)** Pancreas of NaCN+ Naringin 100 mg/kg b.w. treated rats showing the Pancreas with Islets of Langerhans producing normal insulin levels. Magnification- 10X, scale- 100 µm, Field of View (FOV)-2.0 mm, Splitting direction- Transverse.

Table 2. Impact of NaCN and Naringin on Super Oxide Dismutase (SOD) in (U/mg protein)

Groups	Liver	Kidney	Pancreas	Testis
G1 (Control)	14.14072±0.000915	15.60368±0.00176	14.01572±0.00212	13.29392±0.00216
G2 (NaCN)	9.97396±0.002046[a]	9.14615±0.001919[a]	11.205±0.0023[a]	7.96673±0.0015[a]
G3 (NaCN+50mg Naringin)	10.82497±0.002787[ab]	9.86388±0.001848[ab]	11.64432±0.0028[ab]	8.31621±0.00215[ab]
G3 (NaCN+100mg Naringin)	12.79552±0.002389[abc]	11.93748±0.001617[abc]	12.8656±0.0019[abc]	11.4661±0.00213[abc]

Values are shown as mean±SD (n = 6). Superscript letters denote significant differences: [a] vs. G1, [b] vs. G2, and [c] vs. G3 ($p<0.0001$).

Table 3. Impact of NaCN and Naringin on catalase activity (U/mg protein)

Groups	Liver	Kidney	Pancreas	Testis
G1 (Control)	6.844183±0.002519	9.124483±0.002179	12.3763±0.002533	9.914017±0.002568
G2 (NaCN)	4.106683±0.001509[a]	6.36595±0.003207[a]	8.157633±0.001461[a]	4.99515±0.001915[a]
G3 (NaCN+50mg Naringin)	4.933133±0.001712[ab]	6.82685±0.002974[ab]	8.5659±0.003694[ab]	5.334333±0.003032[ab]
G3 (NaCN+100mg Naringin)	5.794233±0.002604[abc]	7.624967±0.002072[abc]	9.646517±0.00251[abc]	7.924933±0.002573[abc]

Values are shown as mean±SD (n = 6). Superscript letters denote significant differences: [a] vs. G1, [b] vs. G2, and [c] vs. G3 ($p<0.0001$).

Table 4. Impact of NaCN and Naringin on Glutathione S Transferase (GST) activity (U/mg protein)

Groups	Liver	Kidney	Pancreas	Testis
G1 (Control)	20.28492±0.0022	22.28252±0.0012	18.32352±0.0014	26.43522±0.0017
G2 (NaCN)	13.4257±0.0025[a]	16.16618±0.0019[a]	10.9734±0.0018[a]	20.2265±0.0027[a]
G3 (NaCN+50mg Naringin)	15.4364±0.0026[ab]	18.89647±0.0014[ab]	11.8772±0.0022[ab]	20.9346±0.0029[ab]
G3 (NaCN+100mg Naringin)	16.5275±0.0018[abc]	19.68722±0.0019[abc]	13.614±0.0016[abc]	23.1561±0.0019[abc]

Values are shown as mean±SD (n = 6). Superscript letters denote significant differences: [a] vs. G1, [b] vs. G2, and [c] vs. G3 (p<0.0001).

Table 5. Impact of NaCN and Naringin on Glutathione peroxidase (GPx) activity (U/mg protein)

Groups	Liver	Kidney	Pancreas	Testis
G1 (Control)	21.61578±0.002068	16.1763±0.002526	15.64635±0.002537	16.89628±0.002136
G2 (NaCN)	16.1259±0.00275[a]	12.12553±0.0033[a]	9.17625±0.00226[a]	13.12603±0.00293[a]
G3 (NaCN+50mg Naringin)	16.78695±0.00184[ab]	12.80477±0.00265[ab]	9.965633±0.00280[ab]	13.61528±0.00221[ab]
G3 (NaCN+100mg Naringin)	17.63637±0.00191[abc]	14.16662±0.00192[abc]	11.69667±0.00247[abc]	14.46623±0.00282[abc]

Values are shown as mean±SD (n = 6). Superscript letters denote significant differences: [a] vs. G1, [b] vs. G2, and [c] vs. G3 (p<0.0001).

Table 6. Impact of NaCN and Naringin on malondialdehyde (MDA) activity (nmol TBARS/mg protein)

Groups	Liver	Kidney	Pancreas	Testis
G1 (Control)	3.86565±0.001162	2.97405±0.000929	3.4217±0.000746	5.103567±0.000927
G2 (NaCN)	5.9885±0.0010315[a]	5.6272±0.00126[a]	6.54335±0.000619[a]	7.335083±0.00244[a]
G3 (NaCN+50mg Naringin)	5.44255±0.0009710[ab]	4.036283±0.00101[ab]	5.534283±0.00176[ab]	6.11418±0.00167[ab]
G3 (NaCN+100mg Naringin)	4.0109±0.00054[abc]	3.10501±0.001007[abc]	4.20276±0.00147[abc]	5.9629±0.00142[abc]

Values are shown as mean±SD (n = 6). Superscript letters denote significant differences: [a] vs. G1, [b] vs. G2, and [c] vs. G3 (p<0.0001).

and a lower dose of naringin (Group 3), catalase activity demonstrated a partial restoration (p<0.0001) compared to Group 2. However, it remained substantially lower than the control. Conversely, the group receiving sodium cyanide and a higher dose of naringin (Group 4) depicted a notable rise (p<0.0001) in catalase activity against Groups 2 and 3, nearly reaching the levels observed in the control group.

The study revealed a significant decrease (p<0.0001) in glutathione S-transferase (GST) activity across all tissues (liver, kidney, pancreas, and testis) in the sodium cyanide-treated group (G2) against the control (G1) as depicted in Table 4. In the group treated with sodium cyanide and low-dose naringin (G3), GST activity showed a partial recovery (p<0.0001) against G2, but it remained markedly reduced compared to the control group. Conversely, the high-dose naringin-treated group (G4) demonstrated a notable increase (p<0.0001) in GST activity compared to Groups 2 and 3, approaching the levels observed in the control group.

A significant reduction (p<0.0001) in glutathione peroxidase (GPx) activity was observed across all tissues (liver, kidney, pancreas, and testis) in the sodium cyanide-treated group (G2) against the control group (G1) as depicted in Table 5. The group treated with sodium cyanide and low-dose naringin (Group 3) showed a partial recovery (p<0.0001) in GPx activity, although it persisted at a markedly reduced level against the control group. In contrast, the high-dose naringin-treated group (Group 4) exhibited a marked improvement (p<0.0001) in GPx activity compared to Groups 2 and 3, with levels approaching those of the control group.

The results displayed a marked rise (p<0.0001) in malondialdehyde (MDA) activity in all tissues (liver, kidney, pancreas, and testis) of the sodium cyanide-treated group (G2) against the control group (G1) as depicted in Table 6, reflecting heightened lipid peroxidation. Treatment with sodium cyanide and low-dose naringin (Group 3) significantly reduced (p<0.0001) MDA activity compared to Group 2, although it remained higher than in the control group. High-dose naringin supplementation (Group 4) led to a notable reduction (p<0.0001) in MDA activity compared to Groups 2 and 3, with values closely aligning with those of the control group.

4. Discussion

Recently, there has been an increasing emphasis on cyanide and its detrimental impacts on both humans and animals. Exposure to cyanide can occur through various means, including mining, industrial activities, smoke inhalation from fires, exhaust emissions from engines, and even tobacco use. The creation of industries that utilise cyanide and gold mines not only resulted in progress, job opportunities, and economic prosperity but also ushered in profound transformations in the environment. These changes manifested as pollution, adverse health effects, and the deterioration of ecological balance[48]. Furthermore, exposure to other cyanide-related compounds like organonitriles, cyanogen, and cyanide-laden pesticides has the potential to induce cyanide toxicity as well. Ingestion of plant-based products containing cyanogenic glycosides is a primary cause of cyanide toxicity in the human population. Many researchers have shown that ingesting cassava root-based foods and breathing in air contaminated with cyanide can result in toxicity. Cyanide is widely recognised as a significant environmental pollutant that is associated with numerous harmful health impacts, including cancer development, liver damage, kidney dysfunction, and disruptions in normal hormonal and reproductive functions[49]. Currently, an authorised antidote for oral cyanide poisoning has not received approval from regulatory agencies responsible for overseeing food and drug safety. Therefore, a viable solution is necessary to counter the effects of oral cyanide exposure[50].

Scientists have shown growing interest in utilising traditional medicines for the effective management and treatment of various illnesses linked to toxicity. These medicines have been found to contain a rich array of phytochemicals, including phenolic compounds, lignins, flavonoids, quinones, alkaloids, amines, betalains, and other photo assimilation products. Notably, these phytochemicals possess a significant amount of free radical scavengers, making them valuable in combating oxidative stress. Consequently, there has been a global shift towards utilising bioactive compounds found in berries, teas, herbs, oil-producing seeds, leguminous beans, fruits, and vegetables[51]. The current research evaluated the mitigating effects of naringin against Sodium Cyanide (NaCN) elicited toxicity in male Wistar albino rats. The results revealed that naringin exposure at doses of 50 and 100 mg/kg substantially ameliorated the effects of NaCN by restoring altered haematological, biochemical, and histological parameters toward normal values. These findings align with prior studies that highlight the antioxidant and protective properties of naringin in oxidative stress-induced conditions[18,19].

The rapid and strong impact of cyanide as an asphyxiant is due to its ability to prevent tissues from utilising oxygen by inhibiting the cellular respiratory enzyme Cytochrome C oxidase. Any sort of exposure causes the absorption of CN into blood, where it binds reversibly with methemoglobin to produce cyanmethemoglobin[52]. Due to the reversible combination, CN gets diffused from the metal complex and inhibits the formation of ATP by preventing cytochrome c oxidase activity by blocking e-transfer and causing anoxia and hypoxia in the tissue[53]. By virtue of its properties, methemoglobin effectively prevents cyanide from reaching cytochrome c oxidase. Consequently, cyanide is gradually released and diffuses out of the red blood cells. Ultimately, it may undergo detoxification through hepatic rhodanese and other sulfur-detoxifying enzyme systems[54]. The detoxification mechanism of the Rhodanese enzyme efficiently converts cyanide to thiocyanate through the catalytic reaction between sulfur and cyanide. Ansell and Lewis[55] approximated that roughly 80% of the ingested cyanide undergoes a conversion process to thiocyanate, a compound that is predominantly excreted through the renal system. Lower doses of cyanide intoxication caused nonsignificant changes in serum biochemistry, haematology and histological parameters. In contrast, higher doses induced significant changes in haematology and serum biochemistry[56].

Excessive cyanide intoxication can result in an increase in cyanmethemoglobin levels within red blood cells, leading to a significant portion of haemoglobin being converted to cyanmethemoglobin. This process ultimately reduces the capacity of red blood cells to carry oxygen. Which leads to the accumulation of oxyhaemoglobin and results in the blockage of oxygen consumption by the tissue. Cyanide poisoning can lead to oxidative damage to erythrocytes, impairing their lifespan and function. This is coupled with the inhibition of erythropoiesis due to cyanide's impact on hematopoietic tissues[57]. The rise in WBC count indicates an inflammatory response triggered by toxin-induced tissue damage. The binding of cyanide to haemoglobin reduces its oxygen-carrying capacity, leading to hypoxia

and anaemia, further emphasising the haematological toxicity of cyanide[58]. In the present study, NaCN exposure significantly altered haematological indices, as evidenced by reductions in haemoglobin, RBC, and platelet levels, along with an increase in WBC and differential counts. Naringin co-administration exhibited a dose-dependent effect in restoring haemoglobin levels towards baseline, increasing erythrocyte count, and substantially decreasing leukocyte count against NaCN toxicity (Table 1). This could be attributed to naringin's antioxidant properties, which protect erythrocytes from oxidative stress-induced hemolysis and enhance the recovery of hematopoietic function. Platelet counts, which were reduced in cyanide-treated groups, were also restored with naringin, indicating its role in preserving thrombopoiesis and overall haematological stability. These results demonstrate that naringin not only mitigates the hematotoxic effects of cyanide but also supports the recovery of blood-making organs, such as the bone marrow and spleen. Such effects highlight its potential as an adjunctive treatment for managing haematological disorders associated with oxidative stress and xenobiotic exposure. This aligns with prior research indicating the naringin's protective effect against arsenic intoxication[59]. In a study, the ingestion of 25 and 50 mg/kg naringenin has been shown to effectively reduce the toxic effects caused by beryllium in blood and serum markers[60]. The observed normalisation of WBC counts in naringin-treated groups indicates a reduction in systemic inflammation (Table 1). This effect is likely mediated by the inflammation-reducing effects of naringin, which suppresses pro-inflammatory cytokines and modulates immune cell functions[29].

NaCN caused detrimental effects on reproductive hormones (FSH, LH, and testosterone) and sperm count, disrupting the hypothalamic-pituitary-gonadal axis and inducing oxidative damage in testicular tissue. Both doses of the naringin treatment group recovered hormonal levels (Figure 1) and sperm count (Figure 2), highlighting its role in protecting testicular function. In prior research, Naringin's antioxidant properties mitigated the lipid peroxidation and preserved Leydig cell function. The concurrent administration of naringin dramatically elevated serum testosterone, FSH, and LH levels[31]. Normal sperm count and function require the presence of both FSH and testosterone, and naringin's role in restoring these levels highlights its protective effects on spermatogenesis. During the toxic insult, histoarchitectural changes in the testes showed degeneration of seminiferous tubules, atrophy of spermatogonia, and enlargement of sperm heads (Figure 7). Various investigations into the male reproductive system have highlighted cyanide as a toxicant for testicular tissue, leading to structural abnormalities such as distortion of seminiferous tubules and germ cell loss[61]. Findings from this study indicate significant improvement in testicular tissue with naringin treatment, including regeneration of spermatogonia, normal germ cell layer, seminiferous tubules with all the different layers of spermatogonia, and increased sperm count (Figure 7).

The liver, being the primary organ to encounter ingested toxins, is exposed to them through its portal blood supply. It serves the crucial function of partially eliminating toxins from the circulation during the initial passage, thereby safeguarding other organs[62]. Within the liver, mitochondrial metabolism and aerobic respiration are pivotal to the physiological activities of hepatocytes, playing a vital role in maintaining homeostasis, facilitating biosynthesis, and regulating amino acid metabolism. In addition, large quantities of cyanide exceed the purification threshold of the rhodanese system in the liver[63]. It implies that cyanide disrupts energy homeostasis and leads to oxidative stress, causing harmful effects on hepatocytes. Cyanide has been found to induce liver and kidney injury in various species like chickens, quails, rabbits, goats, and, rats[64,65]. Histological alterations induced by cyanide have also been documented in pigs[66] and rainbow trout[67]. In this study, the histopathology of the Cyanide-treated group demonstrated hypertrophy of hepatocytes, degenerative central vein, enlarged sinusoids, and cells with pyknotic nuclei in the liver tissue due to the increment in the production and accumulation of ROS. These toxic insults are successfully mitigated by the dose-dependent improvements observed in naringin co-treatment provided with the regenerative central vein, normal hepatocytes, and sinusoids (Figure 4). These findings suggest that the administration of naringin alongside NaCN may confer some protective effect against NaCN-induced liver damage.

Cyanide disrupts mitochondrial function, leading to ATP depletion and ROS overproduction, which contributes to cellular injury[68]. Histological alterations such as hepatocellular necrosis and renal tubular degeneration have also been linked to chronic cyanide exposure (Figure 4 and Figure 5), as demonstrated in previous sub-chronic toxicity studies[67]. Cyanide toxicity significantly impacted liver and kidney function, as

evidenced by elevated levels of AST, ALT, ALP, creatinine, and blood urea in NaCN-treated rats (Figure 3).

The nephrotoxic effects of NaCN in experimental animals are widely recognised. Exposure to cyanide causes significant kidney damage, leading to structural deterioration of both the tubules and glomeruli (Figure 5). Studies suggest that cyanide plays a crucial role in the onset of chronic kidney disease. Kidney function impairment due to cyanide toxicity is typically evaluated by measuring specific serum and urinary biomarkers. Creatine, which is absorbed by skeletal muscles, undergoes phosphorylation by the enzyme creatine kinase, forming creatine phosphate. Both creatine and its phosphorylated form degrade spontaneously and irreversibly into creatinine. Since creatinine levels in urine and serum help assess creatinine clearance, they are commonly used to estimate the glomerular filtration rate (GFR), a critical measure of kidney function. Cyanide toxicity damages cells, leading to the release of creatine phosphokinase, an enzyme involved in the breakdown of phosphocreatine into creatine. The liver converts nitrogenous waste into urea through the urea cycle, a byproduct of protein metabolism. Blood Urea Nitrogen (BUN), which reflects protein breakdown, is excreted as urea via the kidneys and serves as a crucial marker of renal function. Previous studies have shown that rats exposed to cyanide exhibit a marked decline in urinary creatinine and creatinine clearance, while serum BUN and uric acid levels increase[69], which aligns with our observations (Figure 3).

Naringin supplementation significantly ameliorated the effects mentioned above. In the high-dose group (100 mg/kg), serum markers of hepatic and renal function approached normal levels (Figure 3). This is likely due to naringin's ability to scavenge ROS and stabilise cellular membranes, thereby protecting hepatocytes and renal tubular cells from oxidative injury. The flavonoid also enhances mitochondrial function, as observed in its ability to restore energy metabolism and reduce oxidative stress markers[70]. Histological examination corroborated these findings, showing reduced hepatocyte degeneration and glomerular atrophy in naringin-treated groups (Figures 4 and 5). These outcomes align with recent studies demonstrating the hepatoprotective and nephroprotective effects of naringin in toxin-induced injury models. For instance, naringin was shown to alleviate cadmium-induced renal damage by upregulating antioxidant defenses and reducing lipid peroxidation[71]. Similarly, its hepatoprotective potential was validated in acetaminophen-induced hepatotoxicity through modulation of oxidative stress pathways[72,73].

Cyanide exposure has been shown to elevate malondialdehyde (MDA) levels (Table 6) while lowering the activity of essential antioxidant enzymes, including SOD (Table 2), CAT (Table 3), GST (Table 4), and GPx (Table 5), in tissues like the liver, kidneys, testes, and pancreas. These enzymes are essential for safeguarding cells against damage caused by oxidative stress. Therefore, their concentration levels serve as key indicators of the body's ability to neutralise free radicals. MDA is produced when reactive oxygen species (ROS) interact with unsaturated fatty acids in cell membranes. MDA's presence acts as a marker of lipid peroxidation, indicating the degree and intensity of cellular damage[74]. As a result, the levels of CAT, SOD, GST, GPX, and MDA provide an accurate measure of oxidative stress and lipid peroxidation. The findings of this study demonstrate that, in comparison to the control group, NaCN exposure significantly reduced CAT, SOD, GST, and GPX levels in tissue homogenates, while MDA concentrations showed an increase (Table 2-6). Balancing oxidants and antioxidants is essential for cellular stability, as it regulates vital processes like cell growth, differentiation, apoptosis, and necrosis. The results of this study confirm that NaCN disrupts this equilibrium. If ROS are not effectively neutralised, free radicals accumulate and attack polyunsaturated fatty acids within the phospholipid bilayer, leading to lipid peroxidation and increased MDA levels[75]. These findings suggest that oxidative stress is a major contributor to cyanide-induced toxicity. A decline in antioxidant enzyme activity compromises the body's defence mechanisms against oxidative stress, reducing its capacity to eliminate free radicals. As a result, excessive accumulation of free radicals leads to extensive oxidative damage, impairing cellular functions and further deteriorating tissue integrity. Several research findings indicate that naringin helps reduce oxidative stress triggered by environmental pollutants in tissues due to its antioxidant properties[26]. The presence of free hydroxyl groups in its molecular composition plays a key role in its ability to combat oxidative damage and neutralise free radicals. Naringin not only directly counteracts free radicals and lipid peroxides but also promotes the synthesis of non-enzymatic antioxidants while enhancing the function of antioxidant enzymes in living systems. In the present study, both doses of naringin significantly

increased the CAT, SOD, GST, and GPX levels in tissue homogenates, while decreasing the MDA concentrations as compared to the NaCN-treated group (Table 2-6).

This is consistent with the outcomes of Shivanoor and David[35], who documented notable oxidative stress and biochemical alterations in rat sperm due to cyanide exposure. Elevated MDA levels further indicated enhanced lipid peroxidation, a marker of oxidative damage to cellular membranes. In the current investigation, Naringin treatment restored antioxidant enzyme activities in a dose-responsive way. In previous literature, high-dose naringin significantly enhanced antioxidant enzyme activities, suggesting its efficacy in neutralising superoxide radicals and hydrogen peroxide[76]. Increased GPx and GST activities reflect improved detoxification of peroxides and xenobiotics, likely mediated by naringin's upregulation of phase II detoxifying enzymes[77]. The reduction in MDA levels in treated groups further supports naringin's ability to mitigate lipid peroxidation and preserve cellular integrity[78].

In a similar study, Naringin (NRG) supplementation showed notable protective effects against oxaliplatin (OXL)-induced damage in liver and kidney tissues[79]. OXL administration (4 mg/kg b.w.) led to a decline in the activities of key antioxidant enzymes (SOD, CAT, GPx) and an increase in lipid peroxidation, as evidenced by higher MDA levels, resulting in oxidative damage and dysfunction in hepatic and renal cells. However, treatment with naringin (50 and 100 mg/kg b.w.) reversed these effects by restoring the antioxidant enzyme levels and decreasing MDA levels in a dose-dependent manner, highlighting its ability to counteract oxidative damage. Histopathological analysis revealed that naringin treatment reduced OXL-induced hepatocellular injury and renal tubule damage. These results underscore naringin's potential as a therapeutic agent for alleviating oxidative stress, inflammation, and ER stress, making it a promising option for preventing OXL-induced toxicity.

Recent research has highlighted naringin's role in combating oxidative stress in various pathological conditions. For example, naringin improved antioxidant status and reduced ROS generation in a rat model of cisplatin-induced nephrotoxicity[80]. It also demonstrated protective effects against hepatic redox imbalance in a high-fat diet-driven obesity model by enhancing mitochondrial antioxidant enzyme expression[81]. Cyanide-induced pancreatic injury is exacerbated in nutrient-deficient animals[82]. In this study, NaCN treatment depleted beta cells and disrupted pancreatic histology, as evidenced by the vacuolation and degeneration of islets of Langerhans (Figure 6). This may be attributed to redox imbalance and the inhibition of cytochrome C oxidase, which compromises energy metabolism and induces apoptotic cell death. Naringin treatment facilitated the regeneration of beta cells and normalised islet architecture, as evidenced by immunohistochemical analysis. The significant reduction in beta cell vacuolation and necrosis suggests that naringin enhances cellular antioxidant defences and reduces inflammatory responses in pancreatic tissue (Figure 6). This highlights naringin's potential in protecting insulin-producing cells and its role in managing diabetes-related oxidative stress[83]. Additionally, naringin's ability to enhance mitochondrial antioxidant enzyme expression could further support beta cell survival by maintaining cellular redox balance and reducing mitochondrial dysfunction[81]. The strong insulin immunoreactive staining observed in naringin-treated groups indicates increased insulin secretion and preservation of pancreatic function (Figure 8). This aligns with previous reports where naringin improved glucose tolerance and enhanced insulin sensitivity in toxin-induced diabetic models[84]. Such protective effects may have therapeutic implications for mitigating the long-term consequences of oxidative damage to pancreatic beta cells.

Despite the promising results, this study has certain limitations. The sample size, while adequate for statistical analysis, could be expanded in future studies to enhance the robustness of findings. Additionally, the study primarily focused on biochemical and histological markers without delving into molecular pathways involved in naringin's protective mechanisms. Further research using gene expression and proteomic analyses would provide more comprehensive insights into its mode of action. Finally, long-term studies assessing whether the protective effects of naringin persist post-exposure would be valuable in understanding its therapeutic potential in chronic toxicity scenarios.

4.1 Mechanisms of Protection

The protective effects of naringin against NaCN-induced toxicity can be attributed to multiple mechanisms:

- *Antioxidant Activity*: Naringin's radical-scavenging properties mitigate oxidative stress and reduce lipid peroxidation in affected tissues[19].

- *Anti-inflammatory Effects*: By downregulating pro-inflammatory cytokines, naringin reduces systemic inflammation and protects against tissue damage[29].
- *Enhancement of Detoxification Pathways*: Naringin's ability to upregulate phase II detoxification enzymes, such as GST and GPx, facilitates the elimination of toxic metabolites[85].
- *Cellular Regeneration*: Naringin promotes the repair and regeneration of damaged tissues, as evidenced by histological and immunohistochemical findings in the liver, kidneys, pancreas, and testes[20,31].

5. Conclusion

The application of naringin as a treatment option can alleviate hepato-renal, testicular, and pancreatic injury caused by sodium cyanide-mediated toxicity in rats. Naringin also normalises serum markers due to its inflammation-reducing, free-radical neutralising, and anti-mutagenic aspects. These findings prove naringin's protective potential against oxidative stress and toxicity. However, as this study is preliminary, additional studies are required to investigate the molecular processes and possible clinical uses of naringin in managing oxidative stress and cyanide intoxication.

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7. Authors' Contribution

All authors contributed to the study's conception and design. Material preparation, data collection, and analysis were performed by L. H. and M. D. The first draft of the manuscript was written by L. H. and edited by M. D. All authors commented on previous versions of the manuscript. All authors read and approved the final manuscript.

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