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RESEARCH ARTICLE

Pharmacological Evaluation of Polyherbal Formulation for Nephroprotective Activity

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Abstract

The kidney plays an essential role in removing waste products and drugs from the body and maintaining balanced body fluids. It gets affected due to many factors, notably, diabetes and high blood pressure. Nowadays, naturally, derived products are essential in curing various ailments and are safe and cost-effective. The purpose of this study is to assess the toxicity profile and nephroprotective effect of a proprietary polyherbal formulation in Wistar albino rats for gentamicin-induced nephrotoxicity. The Polyherbal formulation was procured from Rumi Herbals Private Limited. Acute toxicity experiments were conducted in Wistar rats using the Gentamicin induced nephrotoxicity model as per OECD standards 423, and the efficacy was assessed using the Gentamicin induced nephrotoxicity model. The formulation was proven safe up to 2000mg/kg orally in an acute toxicity study, with no behavioral abnormalities and no fatality. The gentamicin 80 mg/kg i.p for 7 days induced nephrotoxicity in rats showed a significant ($P<0.05$) increase in the renal parameters and reduction in antioxidant levels compared with day 0. Whereas test drug-treated groups at a low dose (200 mg/kg) and high dose (400 mg/kg) showed significant ($P<0.05$) reduction in elevated renal parameters and improvement in antioxidant levels compared with the disease control group. According to the histopathological interpretation of isolated kidneys, this formulation protects from kidney damage and restores typical kidney architecture. From the results, the proprietary polyherbal formulation has shown effective nephroprotective activity may be due to the presence of secondary metabolites/phytoconstituents. Further investigation is essential to focus on the mechanism involved and standardize the active phytoconstituents responsible for the nephroprotective activity.

Keywords: Acute Toxicity, Antioxidant, Gentamicin, Nephroprotective, Polyherbal Formulation

1. Introduction

Herbal plants are essential in the treatment of a variety of illnesses. Many herbs have been found as sources of critical medicament due to numerous observations and experiments. Medicinal plants have been utilized to treat numerous diseases since prehistoric times. Indian medicinal plants are often used in both traditional (Ayurveda, Siddha, Unani, and Homeopathy) and modern medicine. Herbal treatment is effective, and according to WHO estimates, about 80 % of urbanization relies only on medicinal herbs for health centres. An

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U.S-based analysis demonstrated that about 34% of the population uses one or the other of these systems at least once a year. Herbals are widely used in Germany and France, which account for 39 percent of the $14 billion global retail markets. Plants are responsible for around 70% of manufactured medicines[1].

In Ayurveda, almost 8000 herbal medicines have been described. The Rig Veda (5000 BC) lists 67 medicinal plants, the Yajur Veda 81, and the Atharva Veda 290. (4500-2500 BC). The qualities and applications of 1100 and 1270 species were detailed in the Charak Samhita (700 BC) and Sushrut Samhita (200 BC), respectively. In the Ayurvedic system of medicine and in classical formulations, these are still used to compound drugs. The plant kingdom is a rich conversion of organic chemicals and plays a central role in the growth of innovative medications that are effective in treating various pathological conditions[2]. Over 13000 plants have been examined for their anti-disease and anti-ailment properties worldwide. To treat many ailments, old practitioners employed crude drugs in fresh juice or decoction. Traditional folk healers used therapeutic herbs from established systems of medicine such as Ayurveda, Unani, and Siddha. To treat numerous disorders, they employed crude medications in single or mixed doses[3].

Realizing the huge market potential of Indian traditional medicines, many large industries have set up several units to cater to the increasing demand for these medicinal formulations. Large initiatives that were manufacturing modern medicines have also opened separate herbal divisions to widen their trading market. In addition to manufacturing sastric preparation like 'Lehiyams' and 'churnams', novel dosage formulation of herbal medicine has also been introduced into the market. Thus, with growing market demand and stiff competition, quality assurance of these products is imminent among various manufacturers. Allopathy, which is a popular system of medicines, has some drawbacks. It suppresses the signs and symptoms of the disease alone and does not cure the ultimate cause of the disease. Many drugs in allopathy are also known to cause several side effects. Nephrotoxic incidences and renal failure are on the rise because of the consumption of nephrotoxic drugs and uncontrolled diabetes and hypertension. There is no single drug available currently for nephroprotective activity in the allopathic system of medicine. The polyherbal formulation contains dried whole plants of *Aerva lanata*, dried roots of *Hemidesmus indicus*, the dried stem of *Cretava nurvala*, and dried fruits of *Tribulus terrestris*. This formulation is currently available for urolithiasis and Urinary Tract infections. Since many of the compounds have been reported for nephroprotective activity, this study attempts to evaluate the nephroprotective potential of this formulation.

2. Materials and Methods

2.1 Experimental Animals

The study is being carried out with the agreement of the Institutional Animal Ethics Committee of Madras Medical College, Chennai–03, and this procedure complied with the CPCSEA's national norms (PROPOSAL NO: 03/17/CPCCSEA). Animal experimental laboratory, Madras Medical College, Chennai–03, India, provided the Wistar rats (150 to 200) used in this work.

2.2 Acute Oral Toxicity Study – OECD 423

The polyherbal formulation (PHF), is dissolved in 1% CMC and given to groups of Wistar albino rats via gavage with a feeding needle in a single oral dosage. The control group received the same amount of the vehicle as to the experimental group. The animals were fasted for 12 hours before being dosed. The animals were weighed after the fasting period and then given the test substance at a 2000 mg/kg dose. They were then observed for mortality and behavioral changes for at least 30 minutes, occasionally for the first day, with special attention given during the first 4 hours, thereafter daily for a total of 14 days.

2.3 *In Vivo* Nephroprotective Activity

The rats were put into three groups, each with six rats. Group-1 Served as disease control administered gentamicin 80 mg/kg i.p for 7 days, Group-2 administered gentamicin 80 mg/kg i.p for 7 days and PHF 200 mg/kg for 21 days from day 8 to 28, Group-3 administered Gentamicin 80 mg/kg i.p for 7 days and PHF 400 mg/kg for 21 days from day 8 to 28. On the 0,

8th, and 29th days, blood samples were collected from the retro-orbital sinus in sodium heparin (200 IU/ml) for Haemoglobin evaluation. Furthermore, blood samples were centrifuged for 10 minutes at 3000 rpm to separate serum, then tested for renal parameters, Electrolytes, Lipid peroxidation, and Antioxidants. Finally, on the 29th day, all animals were euthanized with isoflurane (2.5 ml), and the kidney was dissected for histopathological studies.

2.4 Analysis of Biochemical Parameters

2.4.1 Kidney Function Test

2.4.1.1 Serum Creatinine

Tausky used the alkaline picrate technique used to determine serum creatinine levels[4]. 3ml distilled water was used to dilute 1 ml serum. The protein was achieved by the addition of 2ml of 10% sodium tungstate and 2 ml of 2/3 N H2SO4 in drops with steady shaking for 2 minutes. Filtration was done on the solution. 3 mL protein-free filtrate was pipetted out and picric acid 1 mL was added, succeeded by sodium hydroxide. After 15 minutes, the color intensity was measured at 470 nm. The formula was used to calculate the creatinine concentration in the serum, and the results were given in mg/ml. i.e., by dividing test OD by standard OD multiplying with calculation factor.

2.4.1.2 Serum Urea

Natelson's approach was used to calculate urea[5]. 0.1 mL serum was introduced to 3.3 ml deionized water, 0.3 ml 10% sodium tungstate, then 0.3 ml 2/3N sulphuric acid were added, and the solution was thoroughly mixed prior to centrifugation. 1 ml surplus fluid, 0.4 mL diacetylmonoxime, and 1.6 mL sulphuric acid-phosphoric acid mixture were added to 0.4 mL diacetylmonoxime, 1.6 ml sulphuric acid-phosphoric acid mixture. After 30 mins boiling, the mixture was chilled and the spectrometry was observed at 480 nm against a water blank. The formula was used to compute the urea concentration.

2.4.1.3 Serum Uric acid

The serum uric acid concentration was measured using the Fossati *et al.*[6] method. In 300 mL distilled water, 40 g sodium tungstate was dissolved, and 32 mL orthophosphoric acid was added, re-extracted for 4 hours under a vertical condenser. Add 300ml distilled water when it has warmed. To produce a uric acid reagent, dissolve 32gm lithium sulfate monohydrate in 100ml distilled water and add 81ml sodium hydroxide (pH 2.5) to make a uric acid reagent of 10mg pure uric acid in 100ml distilled water as standard. 0.02 mL serum was combined with 1.0 mL uric acid agile and maintained at room temperature for 15 minutes. At 520 nm, the Chroma was measured. The uric acid concentration was determined using the method.

2.4.1.4 Blood Urea Nitrogen

Berthelot technique was used to calculate blood urea nitrogen[7]. To create the test, standard, and blank, 1ml of reagent I comprising urease solution and a combination of salicylate, hypochlorite, and Nitroprusside was added to 10l of serum, 10l of standard urea (40mg/dl), and 10l of pure water, respectively. All of the cuvettes were collected and maintained for 5 minutes at 37°C. Urease is an enzyme that catalyzes the transformation of urea into ammonia and CO_2. The ammonia formed reacts with salicylate, hypochlorite, and Nitroprusside and forms indophenol, a blue-green chemical. The chroma generated is related to the amount of urea in the sample and is quantified at 578 nm. The following formula was used to determine the blood urea.

BUN (mg/dl) = absorbance of test / Absorbance of standard x 40.

2.4.2 Protein Estimation

2.4.2.1 Total Protein

Lowry et al. approach was used to calculate protein[8]. 0.9ml water and 4.5ml alkaline copper reagent together with 0.1ml of properly diluted sample and maintained at room temperature for 10 minutes. After that, 0.5 ml Folin's phenol was applied, and the Chroma produced was measured at 640 nm after 20 minutes. The protein concentration was measured in g/dl.

2.4.2.2 Serum Albumin

Basil T's approach was used to calculate albumin[9]. At room temperature, 0.1 mL serum was combined with 1.0 mL BCG reagent and incubated for 1 minute. In a spectrophotometer, the intensity is measured at 600 nm against a blank. The albumin concentration was measured in mg/dl. Determination of Globulin.

Globulin content was determined by using the following formula,

Concentration Globulin = Concentration Total proteins – Concentration Albumin

2.4.2.3 Determination of A/G Ratio

A/G Ratio was determined by using the following formula

A/G Ratio = Concentration of Albumin / Concentration of globulin

2.4.3 Analysis of Electrolyte Levels

2.4.3.1 Sodium

The colorimetric technique of Maruna & Trinders was used to calculate serum sodium[10]. For the test, 1ml of aggregating reagent was mixed thoroughly with 0.02ml of reference reagent and maintained at room temperature for 5 min before being centrifuged for 2 minutes at 2500 rpm. Each tube yielded around 0.05ml of supernatant. 0.2 mL of tinting agent was added to 3 mL of purified water. 3 mL distilled water, 0.05 mL precipitating reagent, and 0.2 mL coloring reagent make up the blank. The absorbance was measured at 530 nm after incubation at 30 temperature for 5 minutes. The results were expressed as Meq / L.

2.4.3.2 Potassium

The potassium level in the blood was determined using the Maruna technique[11]. 3 ml of boron reagent was added to 0.1 ml of serum. For the standard, 0.1 mL of standard solution and 3 mL of boron solution were applied. The mixtures were thoroughly mixed and incubated for five minutes at room temperature. The color created was colorimetrically measured at 620 nm. Meq / L was used to express the values.

2.4.3.3 Phosphorus

Fiske and Subbarrow's approach was used to calculate inorganic phosphorus[12]. While shaking continually, 0.8ml liquid TCA was applied to 0.2 ml of centrifuging sample. The solution was thoroughly mixed, set aside for a few minutes, and filtered. 0.5 ml molybdate II solution was added to 0.5 ml filtrate. After that, 0.2 mL amino naptholsulphonic acid was added to the mixture. The spectrophotometry was observed at 680 nm after five minutes of standing. The formula for calculating phosphorus content is, multiplying 10 with an absorbance of sample dividing by the absorbance of standard.

2.4.4 Haematological parameter

2.4.4.1 Haemoglobin

The cyanmethaemoglobin technique was used to calculate hemoglobin (Beacon Diagnostic Kit)[13]. A maximum of 20 litres of blood were mixed with 5 mL of Drabkin's solution. Allow sitting for 10 minutes after thorough mixing. With Drabkin's solution as a blank, the absorbance was measured at 540 nm. In the same way, read the absorbance of the standard. The formula was used to calculate the globulin concentration.

Concentration of haemoglobin = (absorbance of test) / (absorbance of standard) x 16.31

2.4.5 Oxidative Parameters

2.4.5.1 Malondialdehyde (MDA/LPO) Estimation

The thiobarbituric acid test technique of Beuge and Aust was used to calculate malondialdehyde[14]. The serum sample was mixed well with 2.0 mL of TCA-TBA-HCl reagent. In a boiling water bath, the temperature was raised for 15 minutes. The flocculants were resuspended for 10 minutes at 1000 g. The sample's absorbance was measured at 535 nm against a blank with no sampling. Using the formula, the concentration of malondialdehyde was estimated.

MDA concentration = (test OD)/ (Standard OD) x (concentration of standard) / (sample taken)

2.4.5.2 Analysis of Enzymatic Antioxidant Parameters

2.4.5.2.1 Superoxide Dismutase (Cu/Zn SOD)

The superoxide dismutase activity was measured using the Kakkar *et al.* technique[15]. With water, 0.5 ml of blood serum was adjusted to 1ml followed by 2.5 mL ethanol. 1.5 mL chloroform and cooled. Then the mixture was centrifuged after being mixed for one minute at 4 °C. Enzyme activity = (C-T)/(C/2) x 60/90 x 1/ (volume of supernatant taken) was used to calculate enzyme activity in the supernatant. 1.2 ml sodium pyrophosphate buffer, 0.01 ml phenazinemethosulphate, 0.3 ml nitroblue tetrazolium, 0.2 ml NADH, properly diluted

enzyme preparation, 0.01ml KCN, as well as water constitutes the test combination in which reactions strats by addition of NADH. The reaction was halted by 1ml glacial acetic acid after 90 seconds of incubation at 30 °C. The reaction mixture was briskly mixed by 4ml of n-butanol. At 560 nm, the chromogen intensity in the n-butanol layer was evaluated against a blank. As a control, an enzyme-free system was used. Enzyme activity was measured as a 50 percent decrease in NBT/min/ml in serum.

2.4.5.2.2 Catalase

Beers and Sizer's approach was used to measure catalase activity[16]. 1.9 mL water and 1 mL hydrogen peroxide reagent were added as substrate, and the mixture was incubated for 5 minutes. The reduction in absorbance was measured at 240nm by adding a 0.1ml sample for 2-3 minutes. The formula was used to calculate enzyme activity. Enzyme activity was expressed as mmol of H2O2 consumed/min/ml in serum.

Enzyme activity = (C-T)/SOD x (concentration of standard)/ (volume of sample taken)

2.4.5.2.3. Glutathione Peroxidase

The efficiency of mitochondrial glutathione peroxidase was measured using Rotruck *et al*[17]. At 37°C incubation the reaction mixture containing 0.2ml EDTA, sodium azide, with H2O2, 0.4ml phosphate buffer along with 0.1ml sample in which it can be halted by 0.5ml TCA. The tubes were subsequently centrifuged at 2000 rpm. 4ml disodium hydrogen phosphate with 0.5ml DTNB to 0.5ml supernatant gives color was observed at 420 nm right away. The activity of GPX was calculated using the formula and represented as a mole of GSH utilized/min/ml in serum.

Activity of GPx = (test OD)/ (Standard OD) X (concentration of standard)/(sample taken).

2.4.5.3 Analysis of Non-enzymatic Antioxidant Parameter

2.4.5.3.1 Reduced Glutathione (GSH)

Moron *et al.* approach's was used to calculate reduced glutathione[18]. The precipitate was cleared by centrifugation after 0.5ml of the sample being precipitated using 1ml of 10% TCA. 1ml of DTNB was mixed with 0.5ml of leftovers and made up to 3ml by phosphate buffer. At 412 nm, the absorbance was measured. The formula was used to calculate GSH. GSH was expressed as µg/ml.

Acitivity of GSH = (Test OD) / (Standard OD) x (concentration of standard)/(sample taken)

2.5 Histopathological Studies

The rats' kidneys were cleansed in iced 10% buffered formalin, fixed in paraffin, and sliced into 5 m thick sections on a microtome. Standard techniques were used to mount parts on glass slides. The slices were stained with Haematoxylin and Eosin and inspected under a microscope at 100 magnifications before being photographed with a light microscope.

2.6 Statistical Analysis

The results were presented as a mean, and standard deviation. One-way ANOVA was used to analyze the data, followed by the Tukey test. The significant variation seen between parameters was related using a one-way analysis of variance (ANOVA). The significance level was set at P 0.05.

3. Results and Discussion

3.1 Acute oral toxicity study – OECD 423

Acute toxicity studies show that when a single dose of a substance is given or several doses are given within 24 hours, harmful effects develop within a short time, usually up to 2 weeks. But here, the polyherbal formulation does not show any behavioral changes, and no mortality was observed here. The NOAEL was found to be 2000mg/kg.

3.2 *In Vivo* Nephroprotective Activity

3.2.1 Analysis of Biochemical Parameters

3.2.1.1 Creatinine

Creatinine is an essential renal biomarker for determining kidney function[19]. Table 1 and Figure 1 show the findings of PHF influence on serum creatinine.

The rats treated with Gentamicin at the dose of 80mg/kg for 7 days showed a marked and significant improvement in the serum Creatinine values in all three groups indicating the presence of nephrotoxicity.

PHF treated for 21 days has shown a substantial decrease in the elevated serum Creatinine level compared with 8th day at both of the dose levels. The decline is more marked at the higher dose levels. This shows the formulation has nephroprotective activity by decreasing the elevated serum Creatinine level.

3.2.1.2 Urea

Protein catabolism produces urea as a by-product. The amino group of amino acids is converted to urea, which is generated in the liver via the urea cycle. Urea is filtered in the glomerulus before being secreted and reabsorption in the tubules. An increase in serum urea is commonly seen as a sign of renal failure, particularly glomerular impairment[20]. Table 2 and Figure 2 show the findings of the influence of PHF on urea levels.

The results show a significant increase in the serum urea level in all the Gentamicin treated groups and a significant decrease in the serum urea level in PHF treated groups at both 200 and 400mg/kg doses.

3.2.1.3 Uric Acid

Uric acid is a chemical formed when the body loses meals containing purines, which are organic substances. The majority of uric acid is absorbed in the blood, filtered by the kidneys, and then excreted. The body might manufacture much more uric acid or not filter it out enough at times. Hyperuricemia is a condition that develops when the body has as much uric acid[21]. The findings of PHF's influence on uric acid levels are shown in Table 3 and Figure 3.

On day 8, the uric acid level in Gentamicin-treated groups is significantly higher than on day 0. The group given 200mg/kg of PHF experienced a significant (P <0.05) drop in serum uric acid levels, whereas the group given 400mg/kg of PHF had a further significant (p 0.001) decrease in uric acid levels.

3.2.1.4 Blood Urea Nitrogen

The breakdown of protein in diets and body metabolism produces blood urea nitrogen, a natural waste product

Table 1. Effect of PHF on serum creatinine

GROUPS	0 Day	8th Day	29th Day
Group – 1	0.64 ± 0.04	1.57 ± 0.05a***	1.92 ± 0.08b***
Group – 2	0.63 ± 0.04	1.43 ± 0.04a***	0.85 ± 0.05b***
Group – 3	0.65 ±0.06	1.76 ± 0.06a***	0.66 ± 0.05b***

a, 8th day vs. 0 day ***P<0.0001 b, 29th day vs. 8th day ***P<0.0001

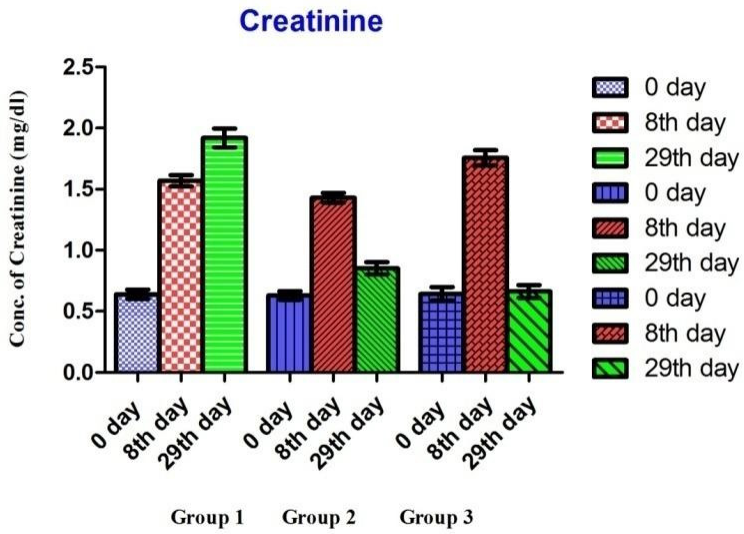

Figure 1. Effects of PHF on serum Creatinine level.

Table 2. Effect of PHF on serum urea

GROUPS	0 Day	8th Day	29th Day
Group - 1	21.8 ± 1.87	46.64 ± 2.86a***	68.60 ± 4.11b***
Group - 2	18.5 ± 1.90	44.82 ± 3.16a***	36.76 ± 4.53b**
Group - 3	20.73 ± 1.52	48.35 ± 5.00a***	24.8 ± 3.79b***

a, 8th day vs 0 day ***P<0.0001, b. 29th day vs 8th day **P<0.001, ***P<0.0001

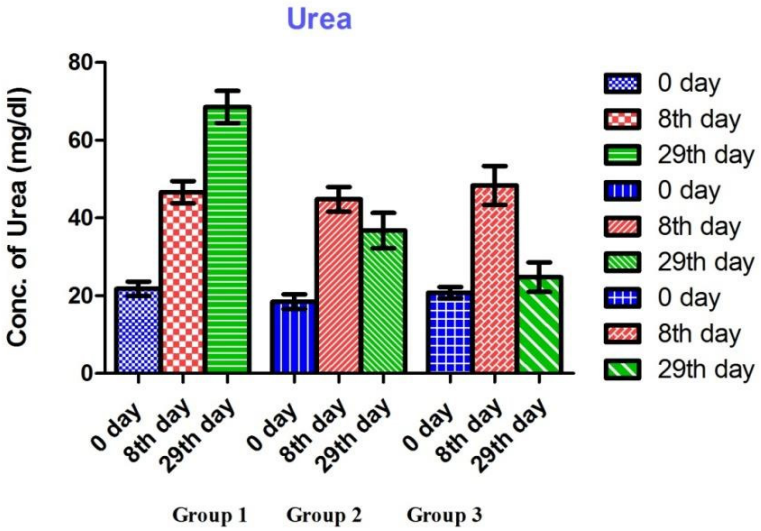

Figure 2. Effects of PHF on serum Urea level.

of human blood. A rise in blood nitrogen levels is azotemia[22]. The effects of PHF on blood urea nitrogen are shown in Table 4 and Figure 4.

The Gentamicin treated groups showed a significant increase in the BUN level compared with 0 day and a significant decrease in the BUN level compared with 8th-day values.

3.2.2 Protein Determination

3.2.2.1 Total Proteins

Proteins are essential building blocks of all cells and tissues. Proteins are necessary for the body's growth, development, and health. The total protein measures the total amount of protein in the blood[23]. The results of the effect of PHF on Total Proteins are given in below Table 5 and Figure 5.

The total protein level in the serum showed a significant decrease in the Gentamicin treated groups and PHF-treated groups significant increase in the total protein level compared with Gentamicin treated groups.

3.2.2.2 Albumin

Albumin is a plasma protein that plays an important role in transporting nutrients and drugs to the target tissues. It maintains oncotic pressure so that blood doesn`t leak from blood vessels. Hypoalbuminemia is a significant independent predictor of acute kidney injury. In kidney dysfunction, mainly tubular necrosis, more amount of albumin is excreted through urine which leads to decreasing albumin level in blood and fails the following functions[24]. The results of the effect of PHF on Albumin are given in below Table 6 and Figure 6.

The results showed a significant decline in the serum albumin level in Gentamicin treated groups. PHF-treated groups show a significant (P< 0.05) increase in the serum albumin level at the dose of 400mg/kg.

Table 3. Effect of PHF on serum uric acid

GROUPS	0 Day	8th Day	29th Day
Group - 1	5.76 ± 0.72	9.26 ± 1.05a***	13.52 ± 1.41b***
Group - 2	6.16 ± 0.87	9.94 ± 0.80a***	10.14 ± 1.25b*
Group - 3	6.86 ± 0.67	10.17 ± 1.03a***	7.65 ± 1.05b**

a, 8th day vs 0 day ***P<0.0001, b. 29th day vs 8th day *P<0.05, **P<0.001, ***P<0.0001

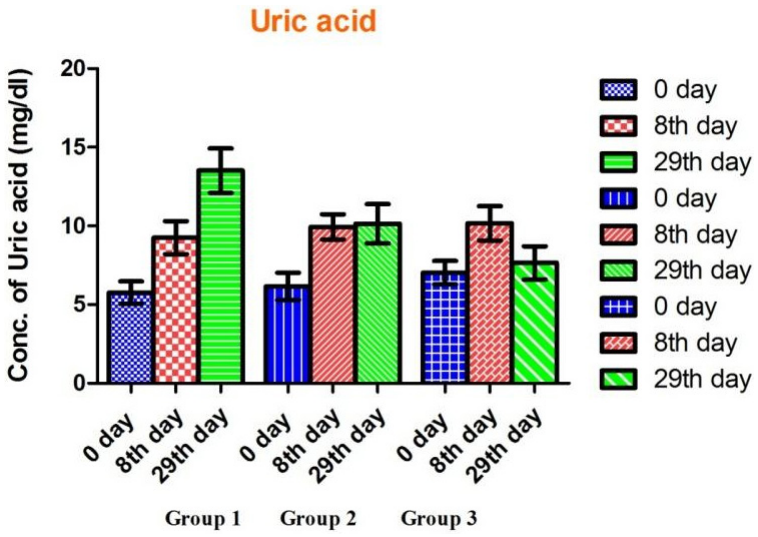

Figure 3. Effects of PHF on serum uric acid level.

Table 4. Effect of PHF on serum blood urea nitrogen

GROUPS	0 Day	8th Day	29th Day
Group - 1	15.43 ± 1.40	32.6 ± 1.50a***	47.82 ± 2.98b***
Group - 2	15.27 ± 1.34	33.21 ± 1.30a***	27.52 ± 2.18b***
Group - 3	13.67 ± 1.30	33.98 ± 2.34a***	18.11 ± 1.69b***

a, 8th day vs 0 day ***P<0.0001, b. 29th day vs 8th day ***P<0.0001

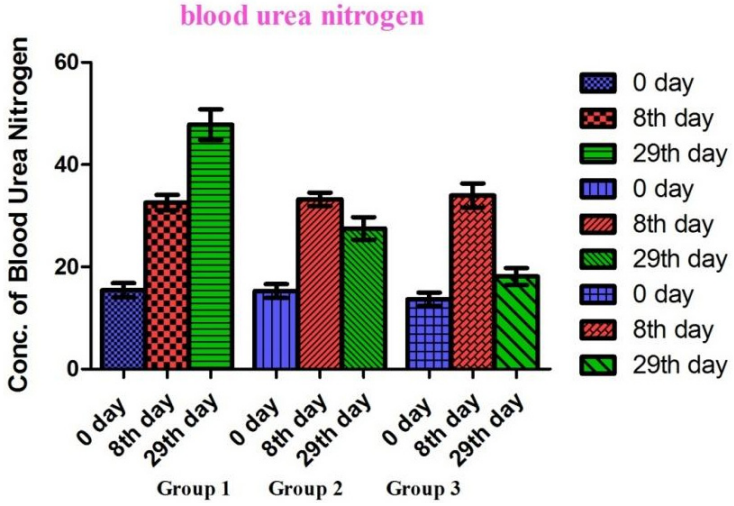

Figure 4. Effects of PHF on serum Blood Urea Nitrogen level.

Table 5. Effect of PHF on total protein

GROUPS	0 Day	8th Day	29th Day
Group - 1	7.32 ± 0.45	4.83 ± 0.53a***	3.64 ± 0.22b***
Group - 2	7.6 ± 0.43	4.24 ± 0.12a***	4.43 ± 0.26b*
Group - 3	6.94 ± 0.31	4.62 ± 0.72a***	5.16 ± 0.12b*

a, 8th day vs 0 day ***P<0.0001, b. 29th day vs 8th day *P<0.05, ***P<0.0001

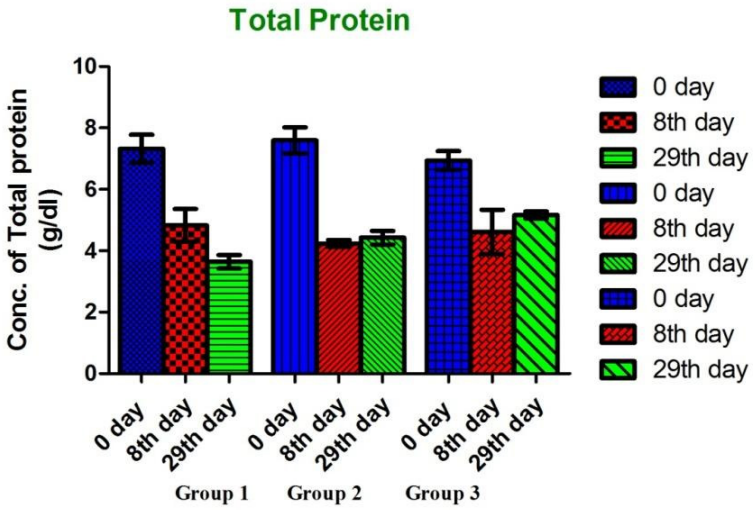

Figure 5. Effects of PHF on total protein level

Table 6. Effect of PHF on albumin

GROUPS	0 Day	8th Day	29th Day
Group - 1	4.02 ± 0.28	1.98 ± 0.13a***	1.32 ± 0.04b***
Group - 2	4.10 ± 0.52	1.84 ± 0.29a***	1.95 ± 0.02bns
Group - 3	4.13 ± 0.57	1.92 ± 0.23a***	2.61 ± 0.27b*

a, 8th day vs 0 day ***P<0.0001, b. 29th day vs 8th day *P<0.05, ***P<0.0001, ns - non significant

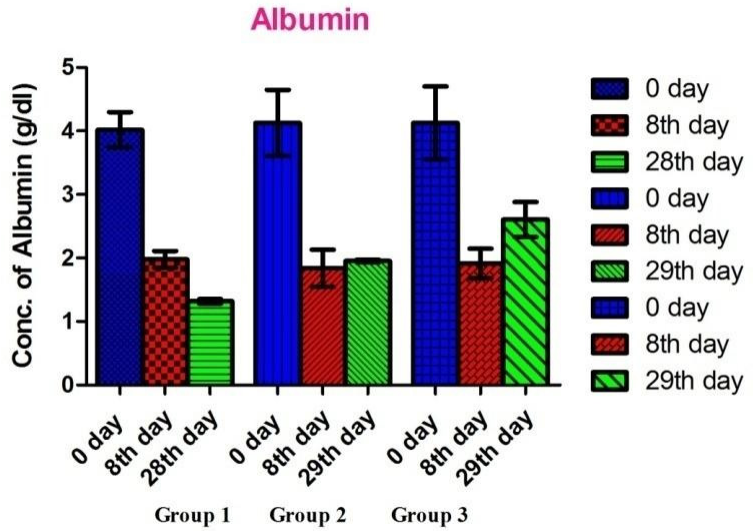

Figure 6. Effects of PHF on albumin level.

3.2.2.3 Globulin

Globulin is a group of proteins that helps fight against infection and transport nutrients. Globulins play an essential role in liver function and blood clotting. It is made in the liver by the immune system. There are four main types of globulins such as alpha 1, alpha 2, beta, and gamma. A low globulin level indicates a sign of kidney disease because more elimination will occur[25]. The results of the effect of PHF are given in below Table 7 and Figure 7.

The results show a significant decrease in the globulin level in Gentamicin treated groups. Treatment with PHF showed no significant increase in globulin levels at both doses.

3.2.2.4 A/G Ratio

The albumin to globulin ratio has been used as an index of the disease state. The average A/G ratio is 0.8 – 2.0. The A/G ratio can be decreased in response to low albumin or elevated globulins. Most of the body's protein is a combination of albumin and globulins. The A/G ratio is helpful in the evaluation of liver and kidney diseases. If the kidneys are defective, protein may be filtered out in the urine, leading to excessive loss of albumin. A value less than 0.8 or 1 is clinically significant that would require further medical evaluation[26]. The results of the effect of PHF on the A/G ratio are given in below Table 8 and Figure 8.

The results show significant increases in the A/G ratio at 400mg/kg compared with Gentamicin treated groups.

3.2.3 Electrolytes analysis

3.2.3.1 Sodium

Sodium is an electrolyte needed for proper nerve and muscle function, and it also helps balance the fluids in the body. Sodium also plays a vital role in regulating hypertension, leading to increases in the filtration process in the glomerulus and damaging the basal membrane of Bowman's capsule, and hardening of fenestrated cells like podocytes. Kidneys can`t effectively remove excess sodium from the blood in renal abnormality[27]. The results PHF is given in below Table 9 and Figure 9.

The Gentamicin treated groups showed a significant decline in the sodium level. The PHF treated groups showed a significant decrease in sodium levels.

3.2.3.2 Potassium

Potassium is an electrolyte that maintains nerve and muscle function. The heart beats at a normal rhythm because of potassium, and it is essential to maintain fluid and electrolyte balance and pH level. The kidney cannot regulate potassium efficiently in case of diminished renal function, which develops hyperkalemia slowly over weeks or months[28]. The results of the effect of PHF on Potassium level are given in Table 10 and Figure 10

The results show a significant reduction in the potassium level in Gentamicin administered groups, and PHF-treated groups showed a substantial decrease in the elevated potassium level at 400mg/kg. There is no significant decrease in potassium level at 200mg/kg. .

3.2.3.3 Phosphorous

Phosphorus is an essential mineral for cell structure and energy. It is about 29% reside intracellular in the bones and less than one percentage circulates in the serum. In kidney dysfunction, fails to excrete the phosphorus and results in a positive phosphorus balance called a hyperphosphatemic state, leading to vascular calcification and loss of tubular functions[29]. The results of the effect of PHF on the phosphorous level are given in Table 11 and Figure 11.

In all the Gentamicin treated groups, there were significantly increased phosphorus levels, and PHF treated groups showed a significant decrease in the phosphorus level at the dose of 400mg/kg, whereas at the dose of 200mg/kg there was no significant decrease in the phosphorous level

3.2.4 Hematological Parameter

3.2.4.1 Hemoglobin

Anemia commonly occurs in people with kidney diseases. Healthy kidneys produce a hormone called erythropoietin. The hormone is reabsorbed into the blood to control or activate specific physiological functions. Erythropoietin stimulates the production of red blood cells in the bone marrow, which then

Table 7. Effect of PHF on globulin

GROUPS	0 Day	8th Day	29th Day
Group - 1	3.3 ± 0.41	2.85 ± 0.12a***	2.32 ± 0.17b**
Group - 2	3.5 ± 0.24	2.40 ± 0.18a**	2.48 ± 0.66bns
Group - 3	2.81 ± 0.46	2.70 ± 0.14ans	2.55 ± 0.22bns

a, 8th day vs 0 day **P<0.001, ***P<0.0001, b. 29th day vs 8th day **P<0.001, ns - non significant

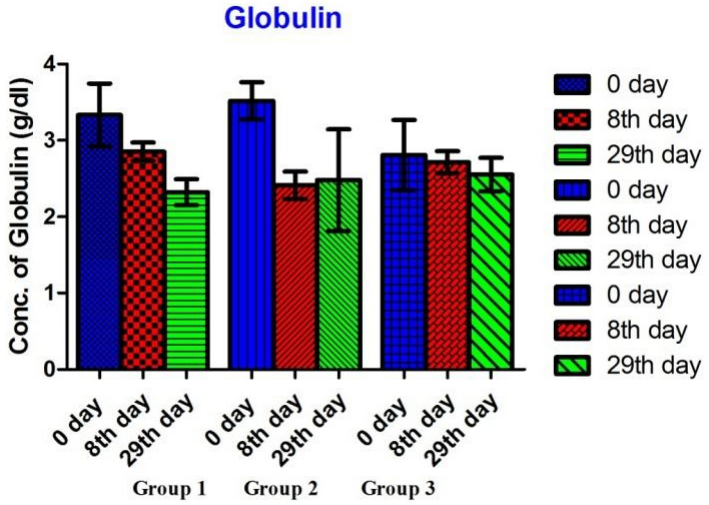

Figure 7. Effects of PHF on globulin level.

Table 8. Effect of PHF on A/G ratio

GROUPS	0 Day	8th Day	29th Day
Group – 1	1.21 ± 0.17	0.69 ± 0.08a***	0.56 ± 0.03bns
Group – 2	1.17 ± 0.15	0.76 ± 0.11a***	0.78 ± 0.07bns
Group – 3	1.46 ± 0.18	0.71 ± 0.10a***	1.02 ± 0.13b**

a, 8th day vs 0 day ***P<0.0001, b. 29th day vs 8th day **P<0.001, ns - non significant

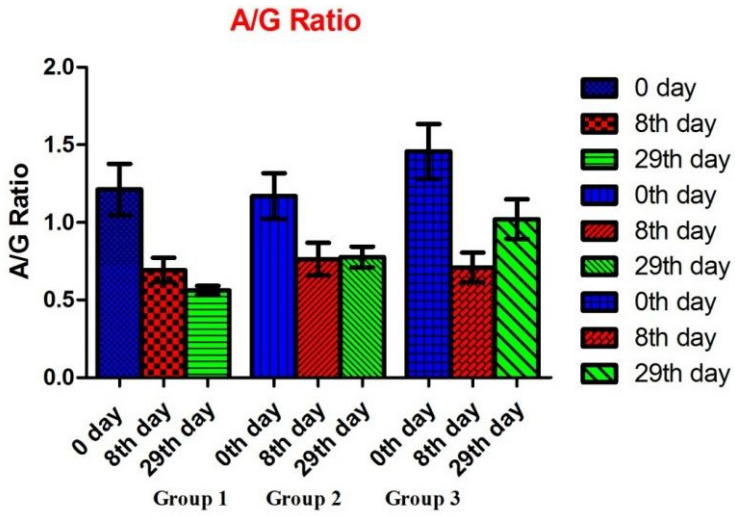

Figure 8. Effects of PHF on A/G ratio level.

Table 9. Effect of PHF on sodium level

GROUPS	0 Day	8th Day	29th Day
Group - 1	141.3 ± 2.26	171.12 ± 2.14a***	198.5 ± 2.11b***
Group - 2	146.6 ± 3.83	177.23 ± 4.47a***	164.6 ± 1.74b***
Group - 3	144.3 ± 4.27	180.02 ± 2.56a***	153.4 ± 2.02b***

a, 8th day vs 0 day ***P<0.0001, b. 29th day vs 8th day ***P<0.0001

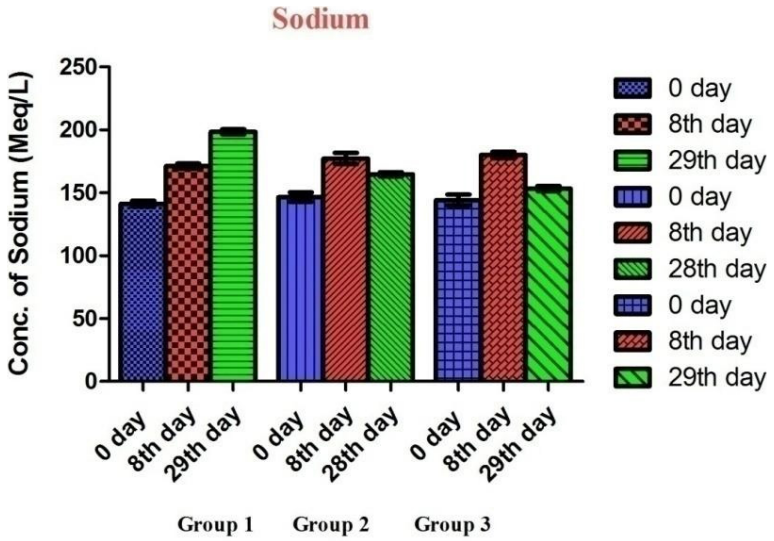

Figure 9. Effects of PHF on sodium level.

Table 10. Effect of PHF on potassium level

GROUPS	0 Day	8th Day	29th Day
Group - 1	4.22 ± 0.20	6.12 ± 0.73a***	7.32 ± 0.51b**
Group - 2	4.07 ± 0.33	6.14 ± 0.44a***	5.87 ± 0.46bns
Group - 3	3.95 ± 0.39	6.17 ± 0.47a***	4.44 ± 0.56b***

a, 8th day vs 0 day ***P<0.0001, b. 29th day vs 8th day **P<0.001, ***P<0.0001, ns - non significant

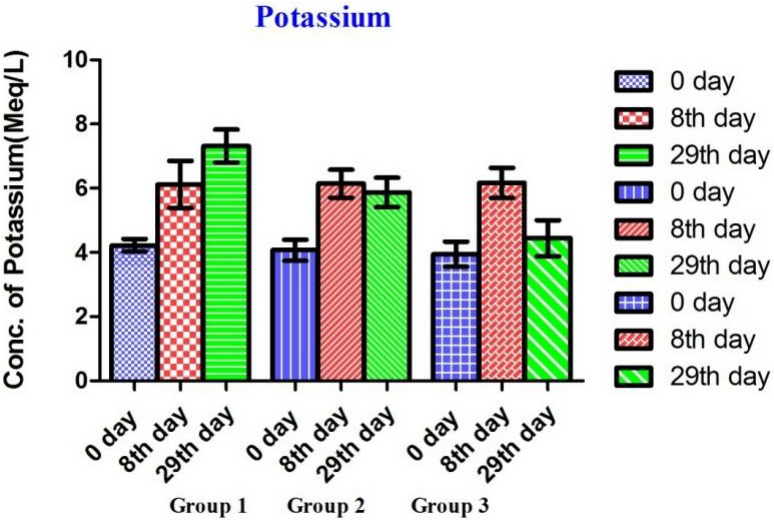

Figure 10. Effects of PHF on potassium level.

Table 11. Effect of PHF on phosphorus level

GROUPS	0 Day	8th Day	29th Day
Group - 1	3.1 ± 0.20	4.2 ± 0.36a***	5.92 ± 0.41b***
Group - 2	3.04 ± 0.20	4.8 ± 0.33a***	5.17 ± 0.28bns
Group - 3	3.14 ± 0.29	4.95 ± 0.43a***	4.16 ± 0.37b**

a, 8th day vs 0 day ***P<0.0001, b. 29th day vs 8th day **P<0.001, ***P<0.0001, ns - non significant

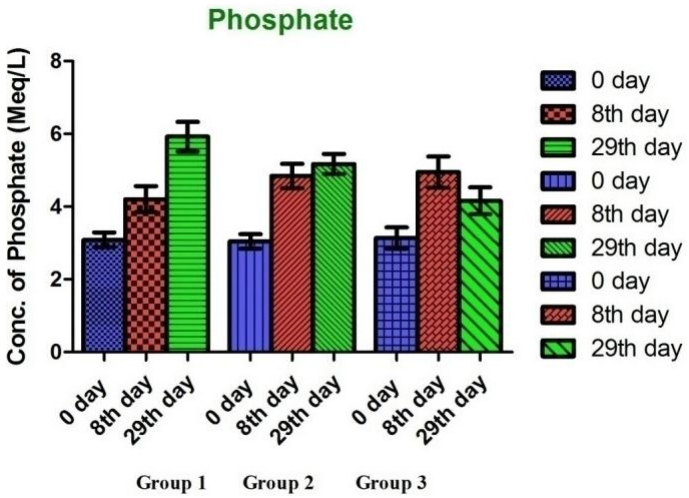

Figure 11. Effects of PHF on phosphate level.

circulate throughout the body. When the kidneys are injured, they produce insufficient erythropoietin. As a result, the bone marrow makes fewer red blood cells, resulting in anemia, which depletes the body's oxygen supply[30]. Table 12 and Figure 12 show the findings of the effect of PHF on hemoglobin levels.

The results of hemoglobin level in Gentamicin treated groups show a significant decrease compared with 0 days in all groups. PHF-treated groups show a significant (P< 0.0001) increase in hemoglobin level in high doses of PHF-treated groups

3.2.5 Oxidative Parameter

3.2.5.1 Estimation of Malondialdehyde (MDA) Levels

The increased amount of Malondialdehyde, a lipid peroxidation marker, in the Gentamicin-induced nephrotoxicity shows enhanced free radical generation[31]. The results PHF effect is given in the below Table 13 and Figure 13.

Gentamicin-induced elevation in malondialdehyde content of serum was significantly protected by PHF treatment in the present study, which indicates attenuation of lipid peroxidation

3.2.5.2 Assessment of Enzymatic Antioxidant Parameters

3.2.5.2.1 Superoxide Dismutase (SOD)

Superoxide dismutase alternately speeds up the dismutation of the superoxide radical into ordinary molecular oxygen or to hydrogen peroxide. It is an important antioxidant defense in nearly all living cells exposed to oxygen[32]. The results are given in the Table 14 and Figure 14.

The results show a significant reduction in the SOD level in Gentamicin treated groups due to the trigger of free radical generation, which is prevented by treatment with PHF. It shows significant SOD levels at the higher dose.

3.2.5.2.2 Catalase (CAT)

Catalase is an enzyme that catalyzes the breakdown of hydrogen peroxide into water and oxygen in all living creatures. It's a crucial enzyme for avoiding reactive

oxygen species from causing oxidative damage to cells[33]. The results of PHF's influence on catalase are shown in Table 15 and Figure 15.

The results show a significant reduction in the Catalase level in Gentamicin-treated groups due to stimulation of free radical generation, which is reduced by treatment with PHF and shows a significant increase in Catalase level in a higher dose of 400mg/kg

3.2.5.2.3 Glutathione Peroxidase (GPx)

The biochemical function of glutathione peroxidase is to reduce lipid hydroperoxides to their corresponding alcohols and reduce free hydrogen peroxide to water. Low levels of GPx observed in type 2 diabetes are associated with macroalbuminuria, which was correlated to the stage of diabetic nephropathy[34]. The Glutathione peroxidase results were given in Table 16 and Figure 16.

The results show a significant decline in the GPx level in Gentamicin treated groups. PHF-treated groups show a highly significant increase in GPx level in higher doses of 400mg/kg.

3.2.5.3 Assessment of Non-enzymatic Antioxidants

3.2.5.3.1 Reduced Glutathione (GSH)

Glutathione can prevent damage to cellular components caused by reactive oxygen species and heavy metals. It

Table 12. Effect of PHF on haemoglobin level

Groups	0 Day	8th Day	29th Day
Group - 1	14.02 ± 0.32	10.74 ± 0.19a***	8.02 ± 0.28b***
Group - 2	13.28 ± 0.53	9.73 ± 0.58a***	9.85 ± 0.69bns
Group - 3	13.19 ± 0.68	9.18 ± 0.44a***	11.20 ± 0.77b***

a, 8th day vs 0 day ***P<0.0001, b. 29th day vs 8th day ***P<0.0001, ns - non significant

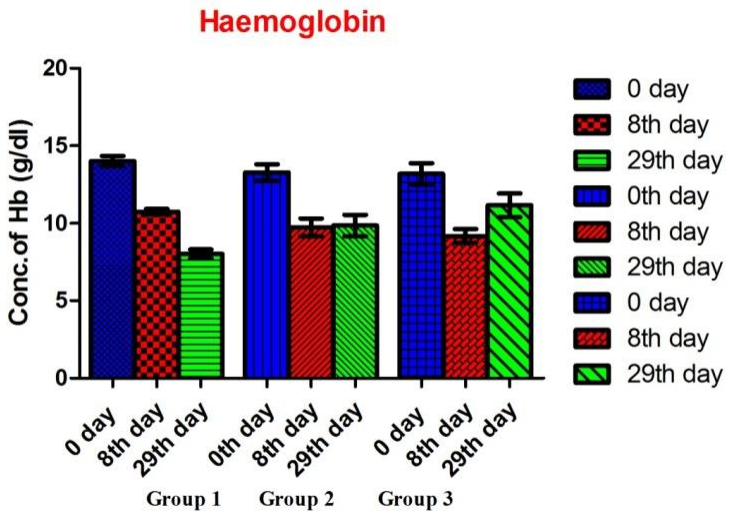

Figure 12. Effects of PHF on hemoglobin level.

Table 13. Effect of PHF on Malondialdehyde (MDA)

Groups	0 Day	8th Day	29th Day
Group - 1	4.52 ± 0.62	10.74 ± 1.32a***	13.56 ± 0.36b***
Group - 2	3.92 ± 0.54	10.82 ± 0.60a***	8.65 ± 0.78b***
Group - 3	4.61 ± 0.45	11.07 ± 1.01a***	6.20 ± 0.58b***

a, 8th day vs 0 day ***P<0.0001, b. 29th day vs 8th day ***P<0.0001

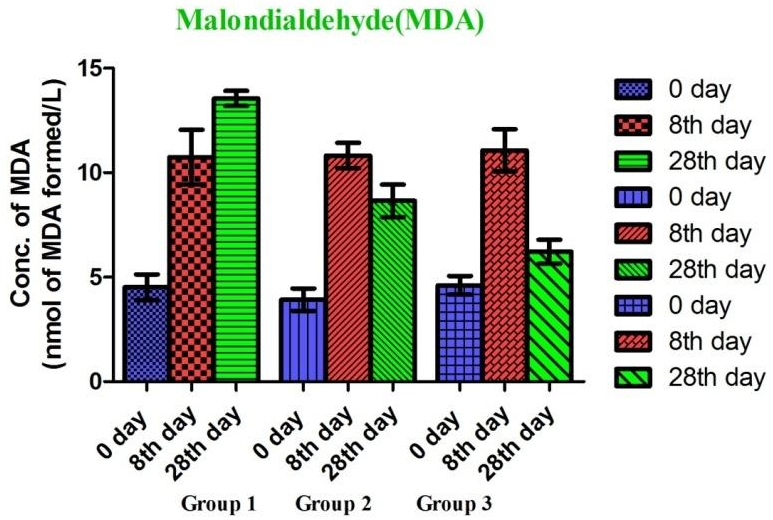

Figure 13. Effects of PHF on Malondialdehyde (MDA) level.

Table 14. Effect of PHF on Superoxide Dismutase (SOD)

GROUPS	0 Day	8th Day	29th Day
Group - 1	8.66 ± 0.31	3.87 ± 0.48a***	3.13 ± 0.48b*
Group - 2	8.25 ± 1.01	4.01 ± 0.51a***	4.85 ± 0.68bns
Group - 3	7.93 ± 0.49	4.63 ± 0.30a***	7.40 ± 0.93b***

a, 8th day vs 0 day ***P<0.0001, b. 29th day vs 8th day *P<0.05, ***P<0.0001, ns - non significant

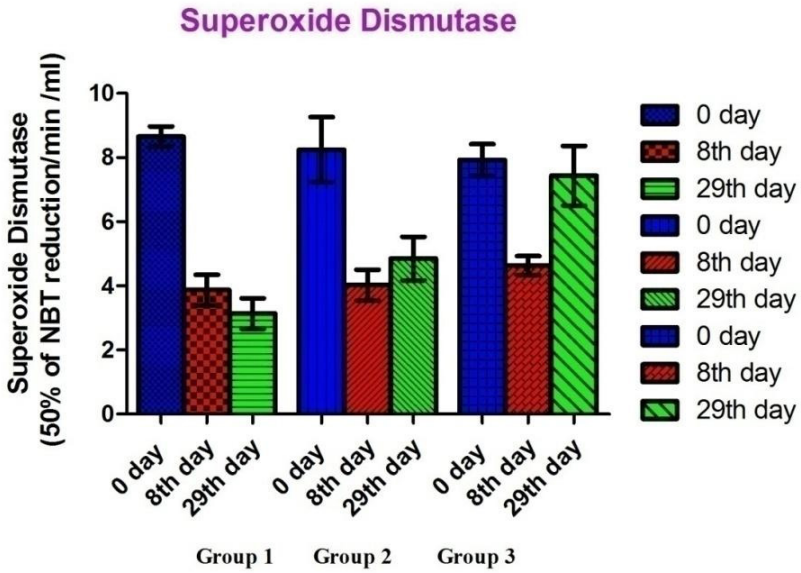

Figure 14. Effect of PHF on superoxide dismutase.

Table 15. Effect of PHF on Catalase (CAT)

GROUPS	0 Day	8th Day	29th Day
Group - 1	10.62 ± 0.95	6.41 ± 0.75a***	4.11 ± 0.42b***
Group - 2	10.87 ± 0.58	6.89 ± 0.83a***	7.60 ± 0.41bns
Group - 3	10.54 ± 1.86	6.12 ± 0.41a***	9.38 ± 0.65b***

a, 8th day vs 0 day ***P<0.0001, b. 29th day vs 8th day ***P<0.0001, ns - non significant

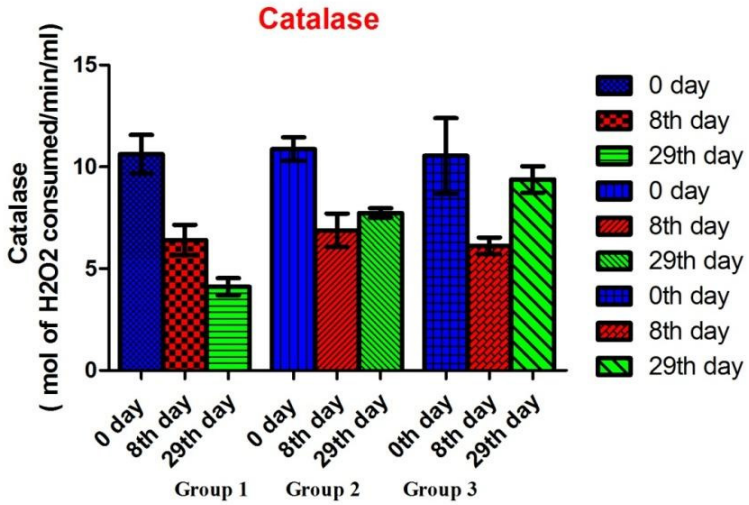

Figure 15. Effect of PHF on catalase.

Table 16. Effect of PHF on Glutathione peroxidase (GPx)

GROUPS	0 Day	8th Day	29th Day
Group - 1	9.98 ± 0.70	7.64 ± 0.43a***	5.32 ± 0.29b***
Group - 2	10.31 ± 0.52	6.98 ± 0.47a***	7.86 ± 0.37b*
Group - 3	9.96 ± 0.37	7.93 ± 0.41a***	9.55 ± 0.52b***

a, 8th day vs 0 day ***P<0.0001, b. 29th day vs 8th day *P<0.05, ***P<0.0001

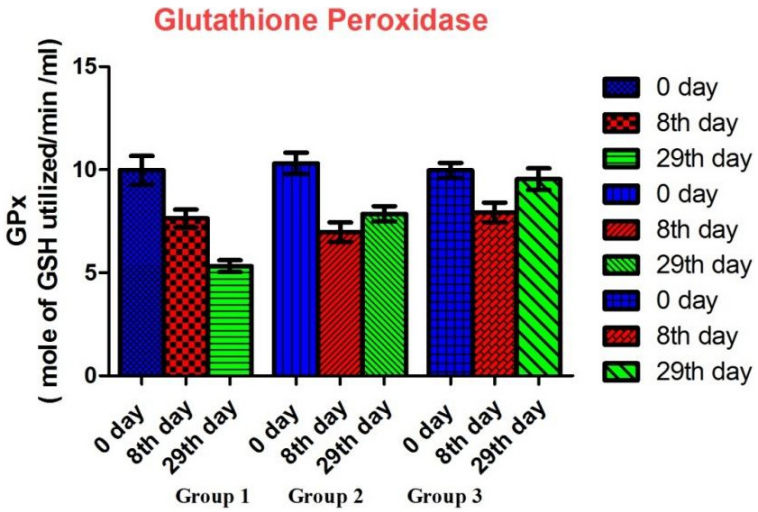

Figure 16. Effect of PHF on glutathione peroxidase.

is a tripeptide with gamma peptide linkage and acts as an endogenous antioxidant. More than 90% of the total glutathione pool in healthy tissues is in the reduced form (GSH)[35]. The results of PHF in Reduced Glutathione are given in the below Table 17 and Figure 17.

The results show a significant increase in the GSH level in PHF-treated groups compared to Gentamicin treated groups.

3.3 Histopathology

The histopathology studies were carried out in all three groups, as photographs are given in Figure 18. The disease control group, the Gentamicin-induced toxic group, showed the congestion in tubular cells and marked destruction in bowman's capsules and glomeruli. Whereas the drug-treated group improved cell regeneration with a well-developed basal membrane, no congestion of renal tubules was observed. It showed that the polyherbal formulation has a protective role in preventing the progression of renal cell loss.

4. Conclusion

The Proprietary polyherbal formulation, analyzed for acute oral toxicity, was observed to have no behavioral

Table 17. Effect of PHF on Reduced Glutathione (GSH)

Groups	0 Day	8th Day	29th Day
Group - 1	7.94 ± 0.60	4.71 ± 0.16a***	4.40 ± 0.42b^{ns}
Group - 2	7.75 ± 0.31	5.08 ± 0.33a***	5.90 ± 0.62b*
Group - 3	8.21 ± 0.52	5.17 ± 0.58 a***	7.40 ± 0.20 b***

a, 8th day vs 0 day ***P<0.0001, b. 29th day vs 8th day *P<0.05, ***P<0.0001, ns - non significant

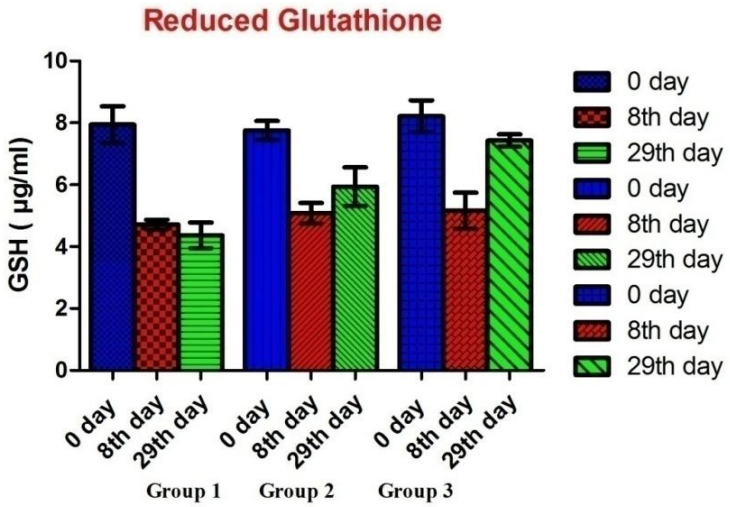

Figure 17. Effect of PHF on reduced glutathione.

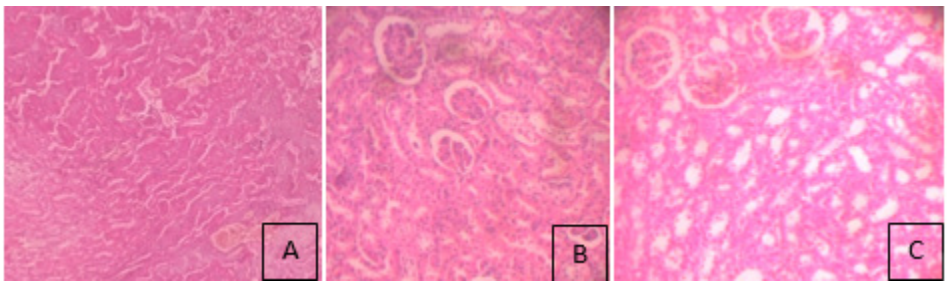

Figure 18. The images show a photomicrograph of kidney **A)** Disease control group, **B)** PHF 200mg/kg, **C)** PHF 400mg/kg.

abnormalities or mortality and was confirmed to be safe up to 2000mg/kg orally. From the efficacy study, renal parameters and protein levels in gentamicin-induced nephrotoxic rats increased significantly compared with day 0, followed by polyherbal formulation treatment, which showed possible protection against nephrotoxicity. Similarly, histological examinations on isolated kidneys suggested that this formulation reversed kidney damage and restored typical kidney architecture. These results suggest that this polyherbal formulation could be effective as a nephroprotective drug in Gentamicin-induced nephrotoxicity. The activity may be due to the presence of secondary metabolites. Further, the amount of active phytochemical constituents have to be standardized for the polyherbal formulation to predict the mechanism of action and clinical trial to be carried out to strengthen the study, which was in progress.

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