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Hepatoprotective Effects of Honey and Curcumin Against Amikacin Induced Liver Injury in Mouse Model

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Abstract

Drug-induced liver injury (DILI) constitutes a major area of concern in clinical medicine with amikacin being a reputed hepatotoxicant. Natural dietary compounds with antioxidants like curcumin and honey could provide a hepatoprotective effect. Here, the efficacy of curcumin and honey as protective agents against amikacin-induced hepatic toxicity was assessed in mouse using biochemical, histopathology, and molecular docking studies. The 24 male Albino mouse (32–38 g, 8–12 weeks) were divided into six groups (n=4). Normal saline was administered to the control group, while the hepatotoxic group received amikacin (35 mg/kg). Treatment groups received curcumin (100 mg/kg, 200 mg/kg) individually or combined with honey (500 mg/kg). Biochemical parameters (ALT, AST, total protein, albumin, and bilirubin) were examined on an Erba biochemical analyzer. Histopathological analysis was done, and molecular docking experiments were carried out to determine curcumin and honey interactions with liver enzymes. The results showed that amikacin treatment highly increased ALT (50.50 ± 47.1 U/L) and AST (84.60 ± 27.7 U/L), but both parameters were significantly low in curcumin and honey-treated groups ($p<0.05$). Histological examination showed reduced hepatic lesions and bile pigment deposition in co-treated groups. Molecular docking studies identified high binding affinities of curcumin (-8.5 kcal/mol) and honey (-7.2 kcal/mol) towards liver enzymes, confirming their hepatoprotective activity. Curcumin and honey successfully prevent amikacin-induced hepatotoxicity by lessening oxidative stress and maintaining liver function.

Keywords: Hepatotoxicity, Curcumin, Honey, Docking, Mouse Model.

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1. Introduction

The liver is an important organ that performs several key physiological tasks, such as detoxification, metabolism, and homeostasis maintenance [1]. In addition to being in charge of blood purification, the synthesis of vital proteins, and the removal of toxins and metabolic waste, it is also

crucial for metabolism of lipids, proteins, and carbohydrates [1]. The liver is structurally composed of four lobes, each of which is made up of smaller functional units termed lobules that help with systemic circulation via the portal vein and hepatic artery [2]. The liver is especially vulnerable to hepatotoxicity because of its crucial role in detoxifying and frequent exposure to a variety of endogenous and exogenous toxicants [3]. Hepatotoxicity is the term used to describe changes in liver structure or function brought on by exposure to chemicals, medications, or environmental pollutants [4]. Because too much exposure to hepatotoxic compounds might overload the liver's capacity to eliminate poisons, it poses a serious threat to global health [5]. Drug-induced liver injury (DILI), which contributes significantly to incidence of acute liver failure globally, is one of main causes of hepatotoxicity [6]. Among hepatotoxic agents, aminoglycoside antibiotics—amikacin in particular—are frequently used to treat bacterial infections that are resistant to several drugs, but they also have side effects, such as hepatotoxicity and nephrotoxicity [7]. Production of reactive oxygen species (ROS), which results in oxidative stress, mitochondrial dysfunction, and hepatocellular injury, is mechanism behind amikacin-induced hepatotoxicity [8]. Hepatotoxicity largely caused by oxidative stress because an imbalance b/w body's antioxidant defense system and ROS generation can result in DNA damage, lipid peroxidation, and protein oxidation, which can ultimately cause hepatocyte necrosis or apoptosis [9].

Natural substances with antioxidant qualities have drawn a lot of interest due to their possible involvement in reducing liver damage, especially in light of the shortcomings of traditional hepatoprotective medications [10]. Because of their well-established pharmacological characteristics, honey and curcumin have stood out among them as potentially effective hepatoprotective drugs [11]. Honey is a natural substance that has anti-inflammatory, antibacterial, and wound-healing properties. It is made up of a complex mixture of sugars, flavonoids, phenolic compounds, and organic acids [12]. It has been shown to have hepatoprotective effects through immune function enhancement, tissue regeneration, and oxidative stress modulation [13]. The active polyphenolic ingredient of turmeric (*Curcuma longa*), curcumin, likewise has strong hepatoprotective, anti-inflammatory, and antioxidant qualities [14]. Studies have shown that curcumin can alter signaling pathways involved in liver cell survival, scavenge ROS, and reduce lipid peroxidation [15]. Curcumin has the potential to be a therapeutic agent, but its low bioavailability and poor solubility have limited its clinical use, requiring the creation of new formulations to increase its effectiveness [16]. With an emphasis on their capacity to reduce oxidative stress and hepatocellular damage, this study attempts to assess the hepatoprotective benefits of honey and curcumin against amikacin-induced liver injury. This research may shed light on the clinical significance of these natural compounds in the treatment of drug-induced liver toxicity and aid in the creation of new therapeutic approaches for hepatoprotection by clarifying the mechanisms by which they achieve their protective effect.

2. Materials and Methods

2.1. Chemicals and Drugs

Curcuma longa (Curcumin) (95% pure) was acquired by an unrefined product supplier and dissolved in distilled water. Amikacin (100 mg injections) was supplied through Sami Pharmaceuticals, and honey came from the Honeybee House at Punjab University in Lahore.

2.2. Animals

Twenty-four (24) healthy male Albino mouse (8-12 weeks old) weighing 32-38g were kept in the animal center at The University of Lahore in Lahore. Mouse were kept at 24 ± 2°C with a 12-hour light-dark cycle and had free access to a normal pellet diet and water.

2.3. Experimental Design

The 24 animals were randomly assigned to six groups (n=4). Before amikacin administration, all groups were given a typical saline-supplemented diet for seven days.

2.4. Treatment Protocol

Table 1 summarizes feeding and drug administration schedule for albino mouse over a seven-day study period.

2.5. Blood Sample Collection

Mouse hearts were used to obtain blood samples for the assessment of oxidative stress and biochemical markers. On the second day, 2 mL of blood were drawn. Following the coagulation process, the samples underwent a 10-minute centrifugation at 4000 rpm. A micropipette used to separate the serum, which was then moved to Eppendorf tubes with labels and kept at -20°C for additional examination.

2.6. Biochemical Parameters

Biochemical markers, such as serum AST (mg/dL), ALT (mg/dL), total protein (mg/dL), albumin (mg/dL), and bilirubin (mg/dL), were estimated using the clear serum that was left over after centrifugation.

i. Alanine Aminotransferase (ALT) Calculation

The liver is the primary location for the enzyme alanine aminotransferase (ALT) [[17]]. Hepatic conditions include cirrhosis, liver cancer, hepatitis, and long-term alcohol misuse causing its levels to increase [18]. L-alanine and 2-oxoglutarate are converted to pyruvate and L-glutamate in an enzymatic process that is mediated by ALT [19]. Lactate dehydrogenase (LDH) catalyzes the reduction of pyruvate by NADH, which lowers absorbance and is strongly correlated with ALT activity [20]. The ALT working solution is made up of two reagents that are made at precise concentrations to allow for the enzymatic reaction. Reagent 1 has pyridoxal phosphate at a concentration of 730 µmol/L, albumin at 0.25% (w/v), malate dehydrogenase (MDH) at 45 µkat/L, lactate dehydrogenase (LDH) at 48 µkat/L, L-alanine at 120 mmol/L, and TRIS buffer at 224 mmol/L, pH 7.8 at 37°C. Reagent 2 contains 2-oxoglutarate at a level of 94 mmol/L and NADH at 1.7 mmol/L. These reagents combine to provide the correct and reliable quantitation of alanine aminotransferase (ALT) activity in biological fluids.

ii. Aspartate Aminotransferase (AST) Computation

The liver, heart muscle, and kidneys are among the tissues that contain aspartate aminotransferase (AST) [21]. A myocardial infarction, liver disorders, or other pathological states are indicated by its increased levels [22]. Oxaloacetate and L-glutamate are created when AST catalyzes the amino group transfer between L-aspartate and 2-oxoglutarate [23]. When malate dehydrogenase (MDH) is present, the

oxaloacetate and NADH combine to generate NAD+ [24]. AST activity is assayed using a two-reagent system. The Reagent 1 has pyridoxal phosphate at 730 µmol/L, L-aspartate at 240 mmol/L, MDH at 48 µkat/L, LDH at 45 µkat/L, and TRIS buffer at 100 mmol/L, pH 7.8 at 37°C. Reagent 2 comprises 2-oxoglutarate at 94 mmol/L and NADH at 1.7 mmol/L. The enzymic reaction continues with AST catalyzing the conversion of L-aspartate and 2-oxoglutarate to oxaloacetate and L-glutamate. The oxaloacetate is reacted with NADH in the presence of MDH to give NAD+, which is measured spectrophotometrically to ascertain AST activity in bio samples.

iii. Total Protein (TP) and Serum Albumin Estimation

The measurement of total protein (TP) and serum albumin was done by an Erba biochemical analyzer [25]. The concentration of total protein was measured by the Biuret method, based on the interaction between peptide bonds and copper sulfate in an alkaline solution that produces a violet-colored complex determinable at 540 nm. The color intensity change was linearly related to the protein concentration. Serum albumin concentrations were measured by the bromocresol green (BCG) method, where albumin complexes with bromocresol green at an acidic pH to form a color complex with an absorbance maximum of 628 nm. The reaction mixture contained bromocresol green (0.12 mmol/L), succinic acid buffer (50 mmol/L, pH 4.2), and sodium dodecyl sulfate (0.15%). The complex was quantified by measuring the absorbance, and albumin concentration was determined from a standard calibration curve.

iv. Total Bilirubin Approximation

Quantification of the total bilirubin was done by the diazo reaction technique, wherein bilirubin reacts with 0.1% diazotized sulfanilic acid to give azobilirubin with a measurable color change. The catalysis was done with 50 mmol/L caffeine-benzoate-acetate to increase the solubility of unconjugated bilirubin. The reaction mixture was incubated at 37°C for 5 minutes and read at 540 nm with a spectrophotometer. Bilirubin concentration was determined from a standard calibration curve (0–2.0 mg/dL) plotted using known bilirubin standards.

2.7. Histopathological Examination

The mouse euthanized on the seventh day after the experiment started, and the liver samples were collected, thoroughly washed with normal saline, and fixed in 10% NBF for 24 hours to preserve tissue architecture. The fixed tissue was dehydrated in a graded series of ethanol (70%, 80%, 90%, and 100%), cleared in xylene, and paraffin-embedded. 3–5 µm thick fragments were cut in a microtome and mounted on glass slides, which were stained with Harris' hematoxylin for 2 minutes and eosin-Y for 1 minute in an attempt to reveal cellular structure. Histopathology was observed with 10×, 40×, and 100× magnification of a light microscope and quantitative scoring for comparison of pathological changes.

2.8. In-Silico Molecular Docking Analysis

2.8.1. Protein Preparation

To prepare them for molecular docking, protein structures associated with liver function tests (LFTs) were extracted from the Protein Data Bank (PDB); ALT (Alanine Aminotransferase) – PDB ID: 2UX6, AST (Aspartate Aminotransferase) – PDB ID: 3K5M, Albumin (Human Serum Albumin) – PDB ID: 1AO6, and Bilirubin UDP-glucuronosyltransferase (UGT1A1) – PDB ID: 2O6L.

2.8.2. Ligand Selection and Preparation

Ligands were chosen for their anti-inflammatory, antioxidant, and hepatoprotective qualities. Curcumin (CID: 969516) is a well-known anti-inflammatory and hepatoprotective drug. Honey contains chrysin, a naturally occurring antioxidant. One flavonoid that has hepatoprotective properties is quercetin. A flavonoid with hepatoprotective and anti-inflammatory qualities is luteolin (CID: 5280445). The PubChem database was used to obtain the ligands' 3D structures, which were then optimized for docking analysis using AutoDockTools (ADT).

2.8.3. Docking Protocol

AutoDock Vina was used for molecular docking. Target protein active sites were identified using computational predictions and literature. PyMOL and Discovery Studio were used to visualize the docking data, which were examined based on binding affinities and interaction characteristics.

2.9. Statistical Analysis

Mean ± standard error (SE) was used to express all results. With SPSS v.16, a one-way analysis of variance (ANOVA) was performed. P-values less than 0.05 were regarded as statistically significant.

3. Results & discussion

3.1. Live Function Tests

i. Alanine Aminotransferase (ALT) Levels

The findings reported in ALT level comparison in the various experimental groups indicate variations in the liver function of enzymes, either indicating hepatotoxic effect or protective effect by the treatments given (Figure 1). The Control group showed the least ALT values (~20 µL/mL). The Amikacin-treated group indicated minimal increase (~25 µL/mL). The Amikacin + *Curcuma longa* (100 mg) group showed values of about 30 µL/mL, and the Amikacin + *Curcuma longa* (200 mg) group showed further increases (~35 µL/mL). The Amikacin + *Curcuma longa* (100 mg) + Honey group had moderate ALT values (~28 µL/mL). The Amikacin + *Curcuma longa* (200 mg) + Honey group had the highest ALT values (~40 µL/mL) with greater variability. Control had an ALT level of 21.25 ± 5.9 µL/mL that serves as a reference value of comparison. The amikacin-alone hepatotoxic control group had a mildly increased ALT value of 26.33 ± 9.60 µL/mL, indicating mild hepatic stress. Experimental Group 1, with 100 mg of *Curcuma longa* (Curcumin), had a moderate increase in ALT to 30.75 ± 6.23 µL/mL, and Experimental Group 2, with an increased dose of 200 mg of Curcumin, had a further increase to 38.00 ± 17.94 µL/mL. This dose-dependent increase indicates that curcumin is possibly devoid of any important hepatoprotective action against amikacin toxicity and even causes elevation of hepatic enzymes at higher doses. Group 3, which received the addition of 100 mg Curcumin to 500 mg Honey, also registered a very mild decrease in the ALT value from 30.25 ± 3.50 µL/mL compared to Group 1, and honey might have had a mild hepatoprotective effect.

But Experimental Group 4 with the highest combination dose of 200 mg Curcumin and 500 mg Honey showed a very high rise in ALT value of 50.50 ± 47.1 µL/mL, which reflects a huge swing in response as can be judged from the very high standard deviation. Statistically, the large error bars for certain groups reflect large subject-to-subject variability, especially in the high-dose combination curcumin-honey group, where the variability is most pronounced. This implies that although some doses might have potential as hepatoprotective medications, over-dosing would result in unpredictable biochemical effects. Official statistical comparison, e.g., ANOVA with post-hoc testing, would be needed to ascertain whether or not these variations were statistically significant. But the observed trend is that curcumin by itself cannot readily reverse amikacin-induced hepatotoxicity and honey at moderate doses may offer some level of protection to the liver. These results are in line with past research that attributed the antioxidative and anti-inflammatory activity of curcumin to suppressing liver toxicity [26].

ii. Aspartate Aminotransferase (AST) Levels

Aspartate Aminotransferase (AST) values between the different groups of experiments reflect variability in the activity of the liver enzymes and reflect hepatic or cardiac stress (Figure 2). The Control group displayed AST levels of approximately 70 Unit/L. The Amikacin + *Curcuma longa* (100 mg) group had a minor increase (~80 Unit/L). The Amikacin + *Curcuma longa* (200 mg) group displayed a further increase (~90 Unit/L). The Amikacin + *Curcuma longa* (100 mg) + Honey group had moderate AST levels (~65 Unit/L). The Amikacin + *Curcuma longa* (200 mg) + Honey group had the highest AST value (~85 Unit/L) with more variability. The control exhibited a value of 71.25 ± 19.56 U/L for AST that acted as a control. The amikacin-treated hepatotoxic control had a mild increase in AST value to 81.66 ± 14.97 U/L, which reflects the mild drug-induced hepatic stress. Experimental Group 1 with 100 mg of *Curcuma longa* (Curcumin) had a maximum data variability in the AST level at 1.02 ± 55.86 U/L. The extremely low mean with the high standard deviation exhibits extremely high variability of data, which could be a result of experimental or biological variance. Group 2 Experimental with a high dose of 200 mg Curcumin exhibited an elevated AST value of 94.25 ± 17.0 U/L, indicating dose dependence and is likely to reflect curcumin may not have potent hepatoprotection when used alone.

Experimental Group 3 received the combination of 100 mg Curcumin and 500 mg Honey had an AST value of 72.75 ± 9.21 U/L, which is similar to that of the control value, which suggests that honey may have mild hepatoprotective activity if combined with curcumin in low dose. Group 4 Experimental, who received a greater dose of 200 mg Curcumin and 500 mg Honey, had 84.60 ± 27.7 U/L levels of AST. This is such a large standard deviation that it indicates enormous individual variation in response to the treatment. ANOVA followed by post-hoc testing would be required to ascertain whether such variations were statistically significant. The trends identified do suggest that curcumin low-dose administered with honey (Experimental Group 3) would have a leveling effect on the AST, where higher curcumin dose-alone (Experimental Group 2) or honey-blended dosages (Experimental Group 4) could have a potential hepatocellular stressing effect. The exceptionally low figure recorded in Experimental Group 1 would be worth probing further for indication of some measure anomaly or outliers. The hepatoprotective effect of honey has been attributed to its ability to suppress lipid peroxidation, enhance endogenous antioxidant defenses, and reduce inflammatory cytokine production [27].

iii. Total Protein (TP) Levels

The amount of total protein (TP) in different experimental groups indicates hepatic function and capacity for protein synthesis (Figure 3). The Control group was around 3.8 g/dL in protein level. The Amikacin group was slightly increased. The Amikacin + *Curcuma longa* (100 mg) and (200 mg) groups were increased in protein levels (~4.5 g/dL). The Amikacin + *Curcuma longa* (100 mg) + Honey and Amikacin + *Curcuma longa* (200 mg) + Honey groups had similar protein levels (~4.6–4.8 g/dL), which indicates a stabilizing effect. The control group indicated a TP value of 3.77 ± 0.32 g/dL as the normal. The hepatotoxic control group (Amikacin-treated) indicated a rise in the level of TP to 4.46 ± 0.65 g/dL, indicating a probable compensatory mechanism or hepatic stress leading to increased protein output. Experimental Group 1 given 100 mg *Curcuma longa* presented a mild increase in the amount of TP to 4.55 ± 0.42 g/dL, and Experimental Group 2 given double that amount, i.e., 200 mg Curcumin, presented TP of 4.40 ± 0.29 g/dL. As may be noted from the above, curcumin supplementation might present a mild stabilizing action over protein synthesis. Experimental Group 3 administered with 100 mg Curcumin and 500 mg Honey also resulted in a level of TP 4.52 ± 0.49 g/dL equal to that treated with amikacin. Group 4 with a higher dose of 200 mg Curcumin and 500 mg Honey has the highest value of TP being 4.72 ± 0.34 g/dL, depicting a visible synergistic effect of honey and curcumin for enhancing the value of protein. Honey contains essential amino acids, vitamins, minerals, and antioxidant compounds that may contribute to enhanced metabolic activity and tissue repair [28]. Moreover, curcumin has been shown to modulate cellular signaling pathways involved in protein synthesis and tissue regeneration. Maintenance of normal or elevated TP levels in treatment groups may therefore indicate preservation of hepatic synthetic capacity despite amikacin-induced stress.

iv. Serum Albumin (ALB) Levels

Serum albumin (ALB) in various experiment groups displays remarkable variation against amikacin hepatotoxicity and *Curcuma longa* (Curcumin) and Honey protective mechanism (Figure 4). The Control group had the lowest albumin levels (~2.3 g/dL). The Amikacin group exhibited a significant increase (~3.2 g/dL). The Amikacin + *Curcuma longa* (100 mg) and (200 mg) groups exhibited slightly lower albumin levels compared to the Amikacin group but were still elevated (~3.0 g/dL). The Amikacin + *Curcuma longa* (100 mg) + Honey and Amikacin + *Curcuma longa* (200 mg) + Honey groups had the highest values (~3.3–3.5 g/dL), implying a possible albumin-augmenting effect of the combination treatment. A control level of 2.27 ± 0.48 g/dL was observed in the control group, which was highly ($p < 0.01$) decreased compared to the hepatotoxic control after the administration of amikacin (3.20 ± 0.50 g/dL). This rise in albumin level in the hepatotoxic group is an expression of a change in hepatic protein metabolism, which can be

attributed to a compensatory mechanism following liver damage. Experimental Group 1 with 100 mg of *Curcuma longa* lowered albumin level (2.87 ± 0.29 g/dL, $p < 0.05$) from that of the hepatotoxic control, which is an expression of a protective effect of curcumin.

Even so, Experimental Group 2, to which was administered 200 mg of *Curcuma longa*, indicated the same amount of albumin at 2.92 ± 0.09 g/dL, but in this case, too, statistical analysis showed $p < 0.05$ and this also speaks volumes about curcumin maintaining albumin further stabilized through its higher dosage. When employed alongside honey as an adjuvant treatment, its influence was shown to be still more active. Experimental Group 3, being treated with 100 mg Curcumin and 500 mg Honey, presented with a statistically significant greater 3.00 ± 0.21 g/dL albumin compared to the control group ($p < 0.01$), showing a synergistic hepatoprotective action of curcumin and honey. Maximal improvement was seen in Experimental Group 4 on taking 200 mg Curcumin and 500 mg Honey with a recorded value of 3.27 ± 0.15 g/dL of albumin significantly ($p < 0.01$) superior compared to the curcumin low-dose group and the control groups. The findings justify that *Curcuma longa*, especially supplemented with honey, exerts hepatoprotective activity at serum albumin level against amikacin-induced hepatic damage [29]. Statistical significance ($p < 0.05$, $p < 0.01$) suggests that curcumin and honey supplementation can prevent liver injury and induce protein synthesis to ensure albumin homeostasis.

v. Total Bilirubin (TB) Levels

Total bilirubin concentrations (TB) in various test groups indicate hepatotoxicity of amikacin and protection by *Curcuma longa* (Curcumin) and Honey (Figure 5). The Control group registered the lowest bilirubin levels (~0.1 mg/dL). The Amikacin group registered a rise in bilirubin levels (~0.25 mg/dL), indicating probable liver stress or damage. The Amikacin + *Curcuma longa* (100 mg) and (200 mg) groups had minimally decreased bilirubin levels (~0.15 mg/dL) relative to Amikacin group, suggesting a protective effect. The Amikacin + *Curcuma longa* (100 mg) + Honey and Amikacin + *Curcuma longa* (200 mg) + Honey groups had comparable levels of bilirubin (~0.15 mg/dL), which also hints that *Curcuma longa* combined with honey could aid in preventing elevation of Amikacin-induced bilirubin. Control group baseline total bilirubin level of 0.10 ± 0.04 g/dL was significantly raised to 0.20 ± 0.07 g/dL after treatment with amikacin in the hepatotoxic control group. The increased level ($p < 0.05$) shows damage to liver as it is an indicator of hepatic damage and perturbation in bilirubin clearance. Therapeutic classes indicate Curcuma 100 mg *Curcuma longa* (Group Experimental 1) reduced level of total bilirubin to 0.11 ± 0.11 g/dL, which is moderate hepatoprotection, albeit with a relatively higher standard deviation.

A dose of 200 mg *Curcuma longa* (Experimental Group 2) further reduced bilirubin to 0.09 ± 0.08 g/dL, close to the normal control value. These decreases were not significantly different from a high probability level ($p > 0.05$). The combination of curcumin and honey had an increasingly better effect. Experimental Group 3, which was given 100 mg *Curcuma longa* with 500 mg Honey, also had a value of 0.12 ± 0.03 g/dL, an indication of enhanced hepatoprotection over curcumin. Likewise, 200 mg *Curcuma longa* with 500 mg Honey (Experimental Group 4) also had a value of 0.122 ± 0.49 g/dL, but the large standard deviation indicates response variability. Together, these findings suggest that the *Curcuma longa*, especially in higher doses and with honey, prevents amikacin-induced liver injury by reducing the bilirubin level to almost normal [29]. Although changes reflect a hepatoprotective effect, a loss of statistical significance is caused, which necessitates the replication using larger sample sizes.

3.2. Histopathological Evaluation

The histopathological analysis of liver tissue also demonstrated significant group differences. The histology of liver in the vehicle control group was normal liver structure with normal hepatocytes, central nuclei, and no evidence of bile pigment deposits, inflammation, or cellular swelling. It ensured that liver was healthy with active Kupffer cells and liver lesion-free. There were, nonetheless, under category of amikacin-induced hepatotoxicity significant liver injury with widespread bile pigment deposition, extreme hepatocellular swelling in majority of areas, and necrotic debris. Kupffer cell activation and nuclear degeneration further substantiated extreme hepatic injury with nearly 80% participation of liver, a very extreme degree of inflammation (4.5/5), and massive cellular swelling. Curcumin 100 mg/kg treatment (Experimental Group 1) led to only minimal restoration of architecture of liver. Although some relief from swelling & bile pigment deposition was present, histopathologic assessment still revealed evidence of widespread hepatic lesions and inflammation with nearly 70% tissue involvement. Boosting the dose of curcumin to 200 mg/kg (Experimental Group 2) was not found to have any other significant protective effect, as bile pigmentation deposit, hepatocellular edema, and inflammation were present even then, covering approximately 65% of tissue. Findings suggest that curcumin administered alone in augmented doses is not found to exert significant hepatoprotective action against amikacin-induced damage [30].

More improvement was observed when honey and curcumin were combined at 500 mg/kg honey and 100 mg/kg curcumin (Experimental Group 3). Hepatic lesions and deposition of bile pigments persisted, but the degree of liver injury was less than that in the amikacin-induced and curcumin-alone groups. Histological examination revealed decreased hepatocyte swelling and inflammation, bile pigment deposition in approximately 50% of tissue, and an inflammation score of 3/5. This indicates that honey can bring about a synergistic action with curcumin and provide a moderate degree of hepatoprotection [31]. The maximum hepatoprotective effect was in Experimental Group 4 treated with 500 mg/kg honey and 200 mg/kg curcumin. Tissue from this group's liver contained only slight edema, lower hepatic lesions, and middle total liver injury. The pigments of the bile were decreased to approximately 30%, while hepatocyte edema and inflammation were also lesser in amount with an inflammation rating of 2/5. This means that the combination of curcumin at 200 mg/kg with honey at 500 mg/kg provides the maximum level of hepatoprotection, significantly reversing amikacin-induced liver damage and lowering hepatic injury (Figure 6). The Vehicle Group shows normal hepatocyte and Kupffer cell pattern with normal architecture of the liver. The AMK-Induced Hepatotoxic Group shows the bile pigments deposition, swelling of the hepatocytes, and tissue damage.

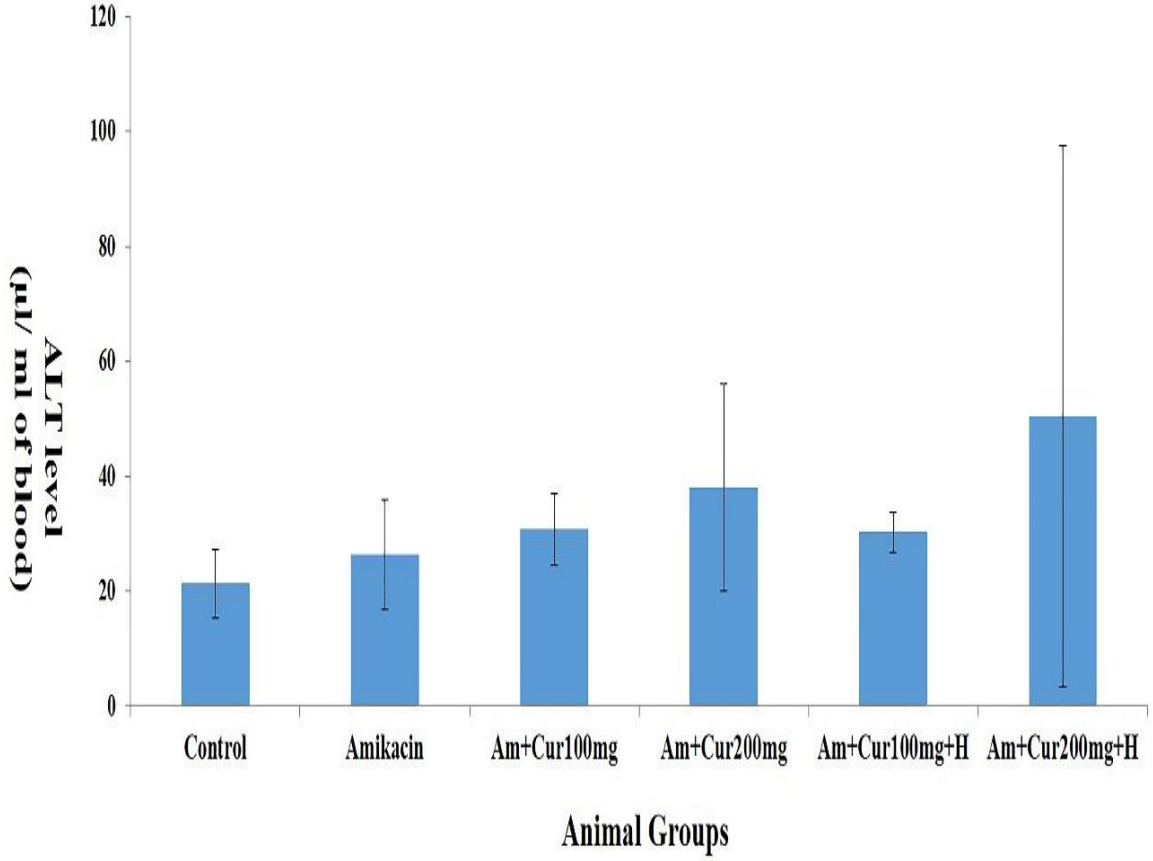

Figure 1: Serum ALT values (Mean ± SE, µL/mL of blood) in various experimental groups.

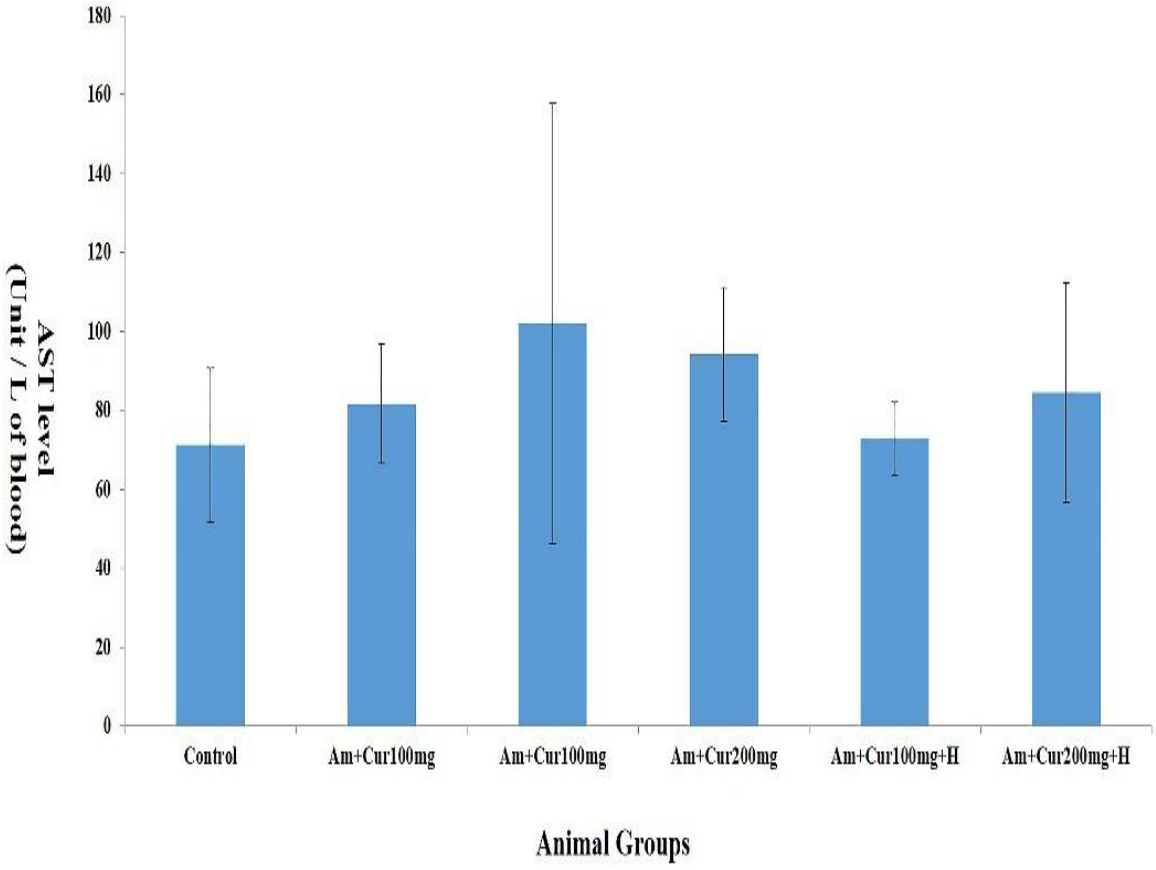

Figure 2: Serum AST levels (Mean ± SE, Unit/L of blood) in various experimental groups.

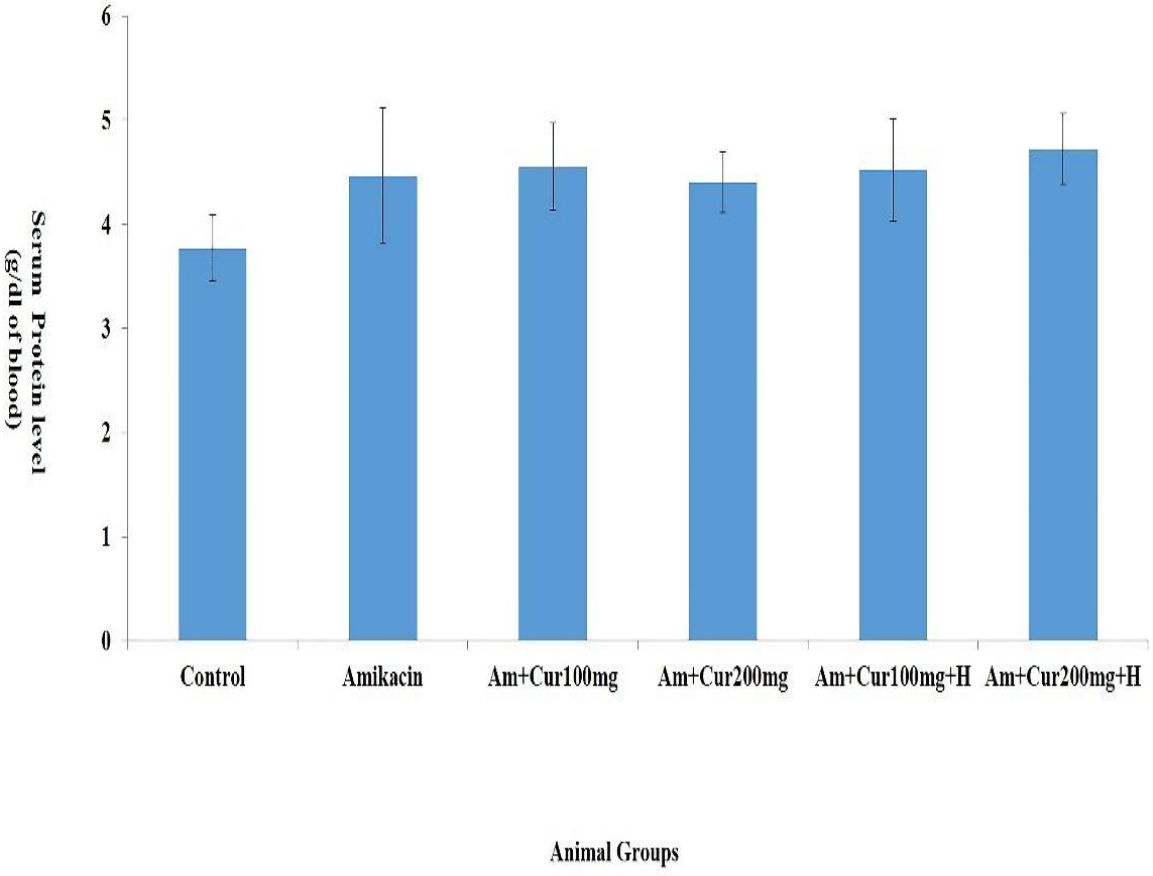

Figure 3: Serum Protein levels (Mean ± SE, g/dL of blood) in various experimental groups.

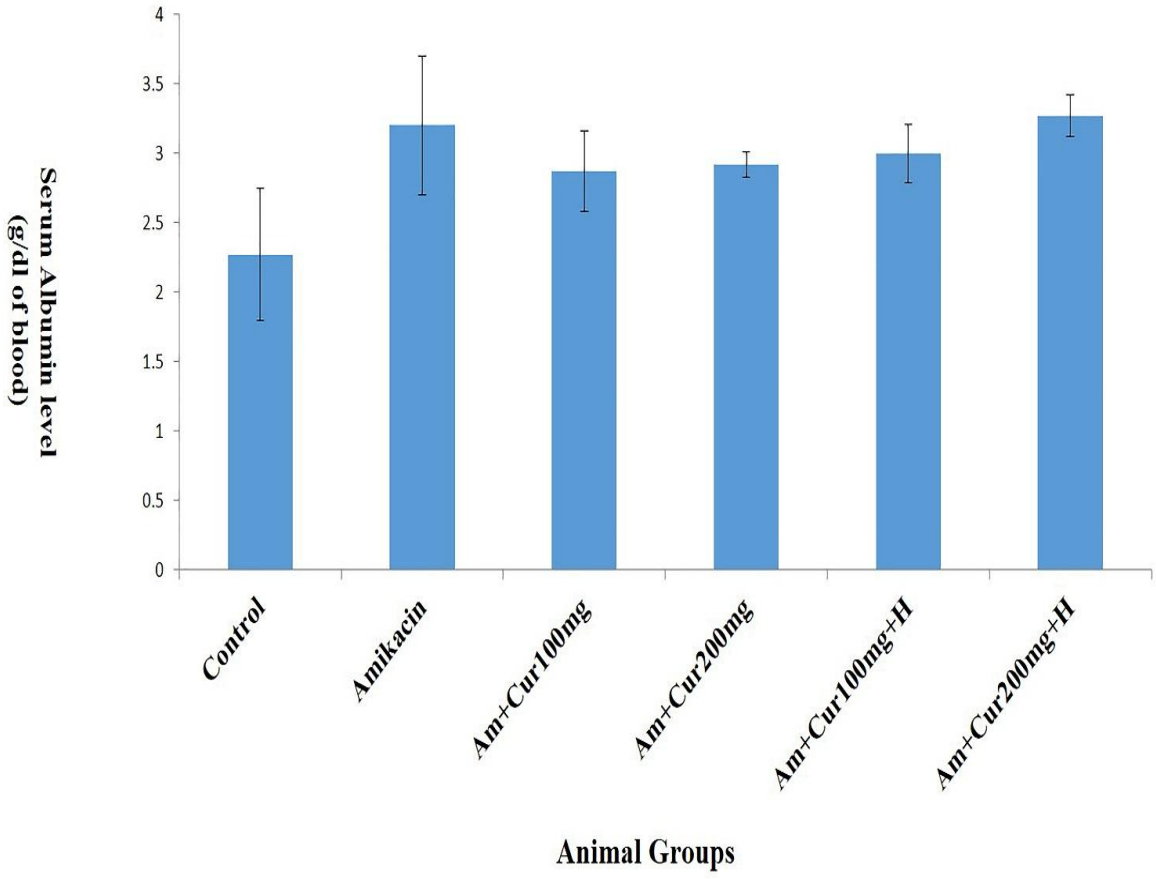

Figure 4: Serum Albumin levels (Mean ± SE, g/dL of blood) in various experimental groups.

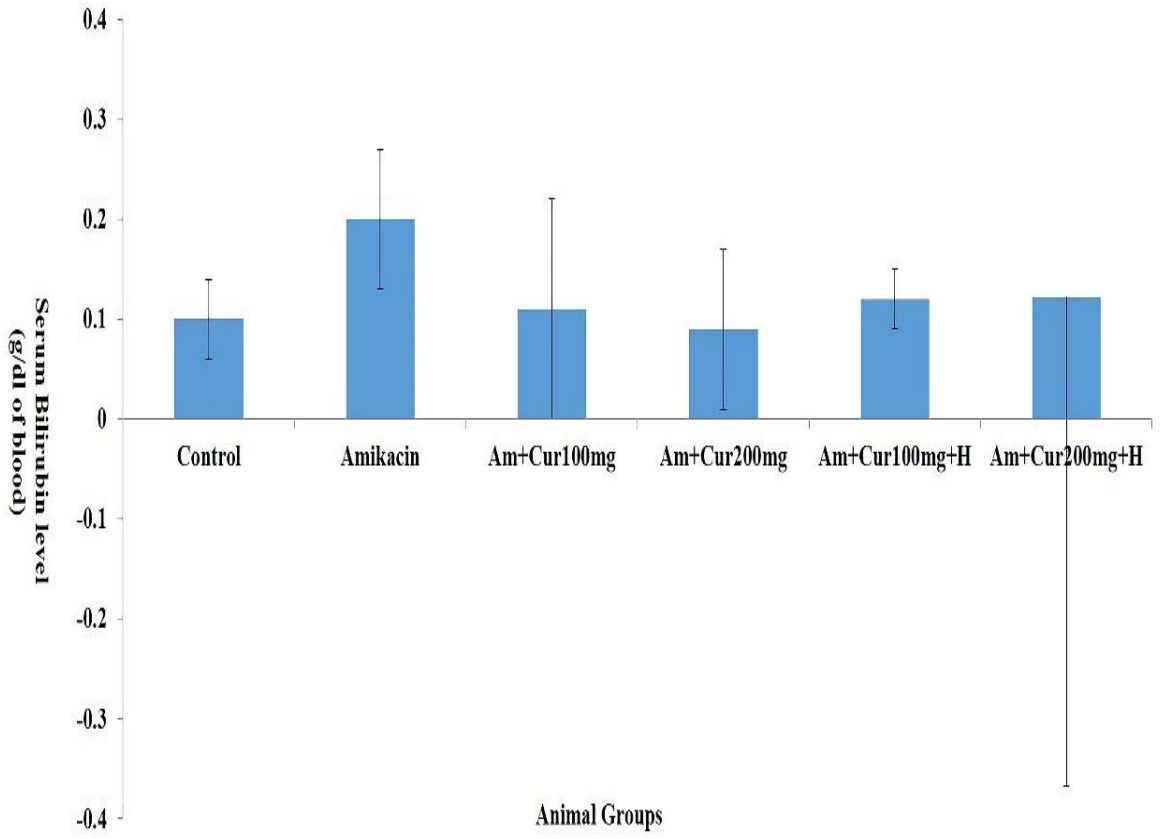

Figure 5: Serum Total Bilirubin Levels (Mean ± SE, mg/dL) in Control and Experimental Groups.

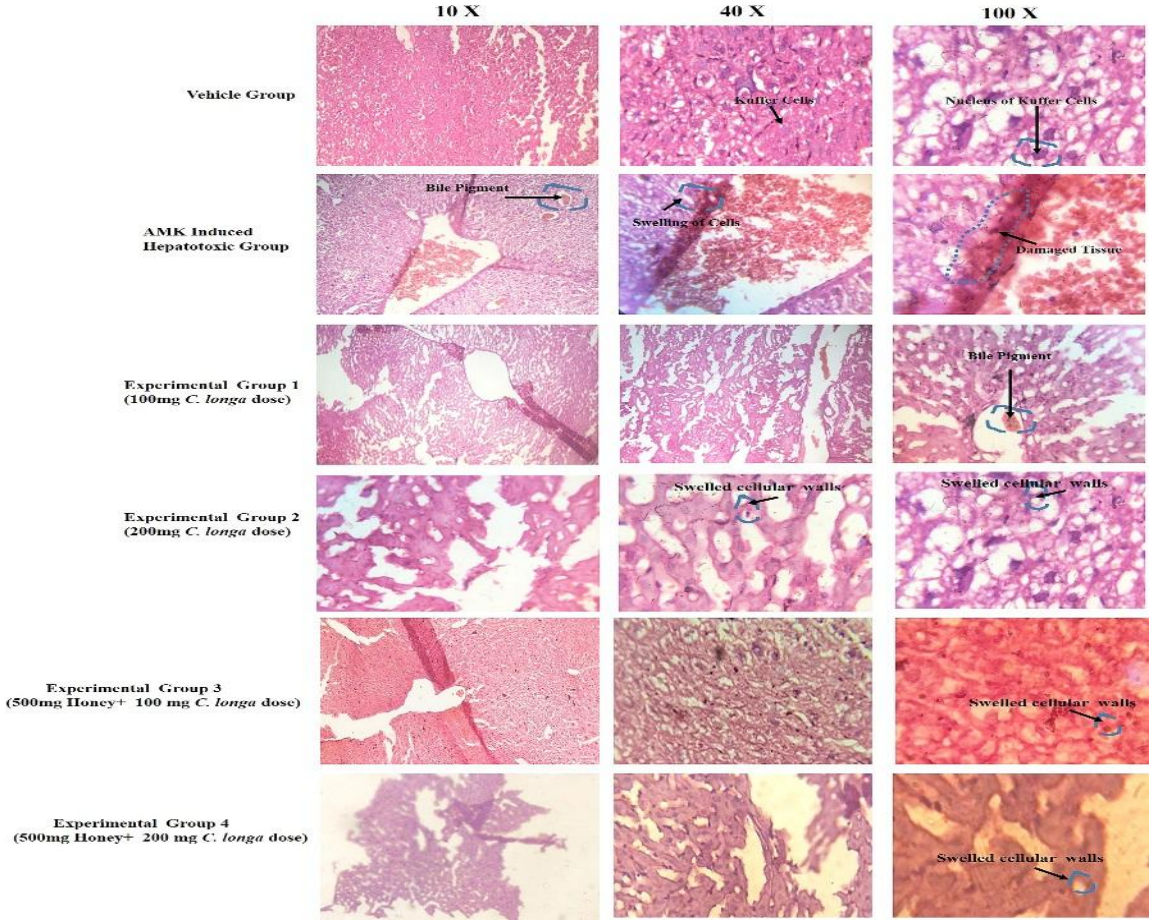

Figure 6: Histopathological study of liver tissues under 10X, 40X, and 100X magnifications from different experimental groups.

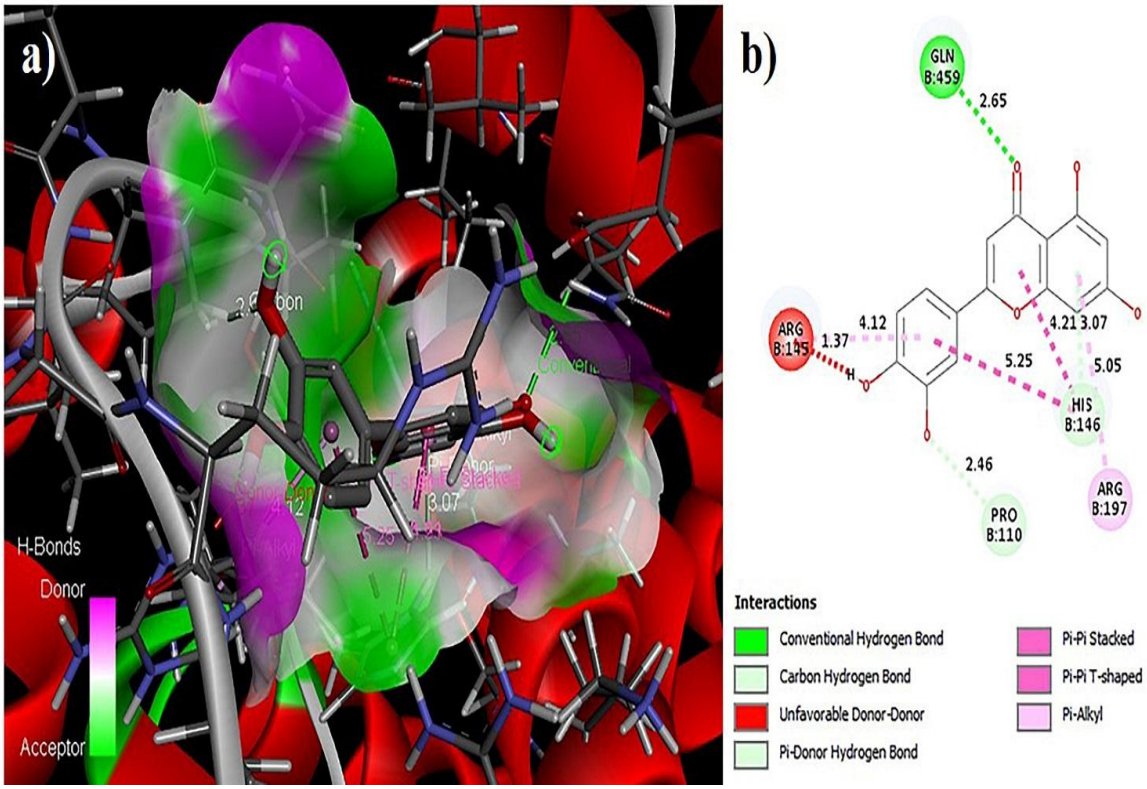

Figure 7: a) 3D docking visualization of luteolin (CID: 5280445) bound to bilirubin UDP-glucuronosyltransferase (UGT1A1) (PDB ID: 2O6L). The molecular surface highlights key interaction sites, with ligand atoms in stick representation. **b)** 2D interaction diagram of luteolin with bilirubin UDP-glucuronosyltransferase (UGT1A1) (PDB ID: 2O6L). Various interactions, including hydrogen bonds (green), pi-stacking (pink), and carbon hydrogen bonding (light red), are visualized along with bond distances.

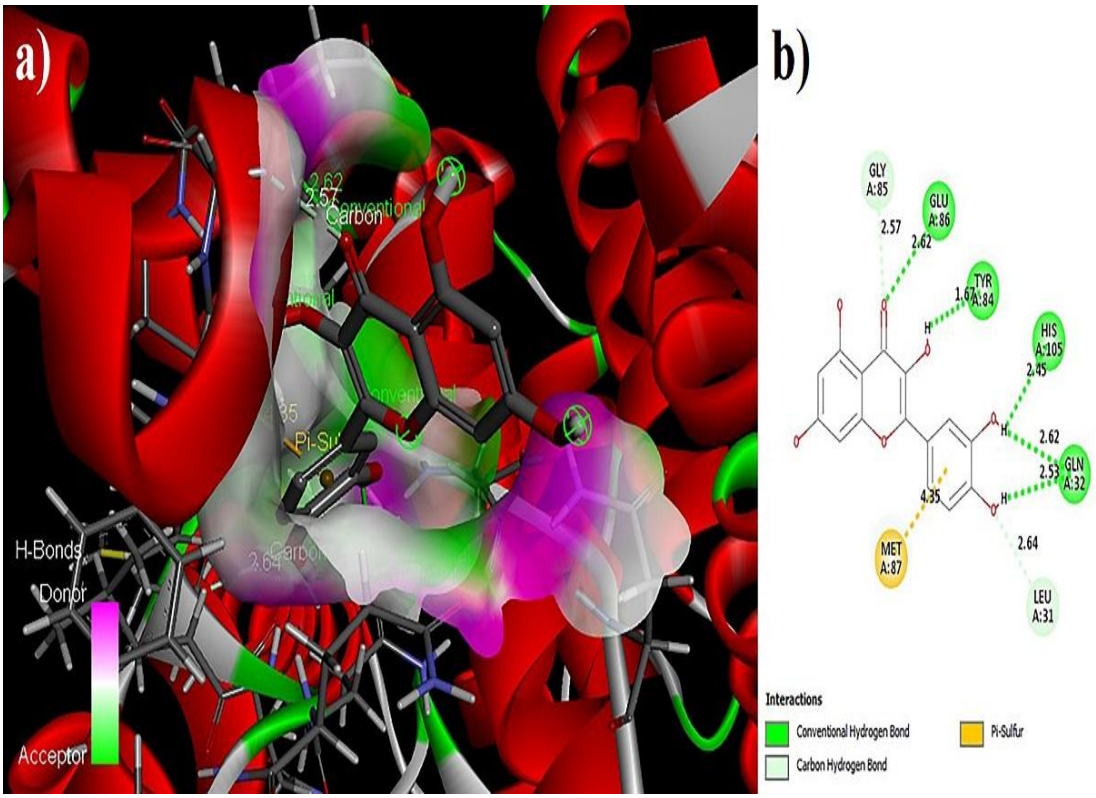

Figure 8: a) 3D docking visualization of hesperetin (CID: 5280343) bound to peroxisome proliferator-activated receptor gamma (PPAR-γ) (PDB ID: 1BM0). The molecular surface representation highlights binding site interactions, with ligand atoms displayed in stick format. **b)** 2D interaction diagram of hesperetin with peroxisome proliferator-activated receptor gamma (PPAR-γ) (PDB ID: 1BM0). The figure shows hydrogen bonds (green), pi-sulfur interactions (yellow), and carbon hydrogen bonding (light green), indicating a stable ligand-receptor interaction.

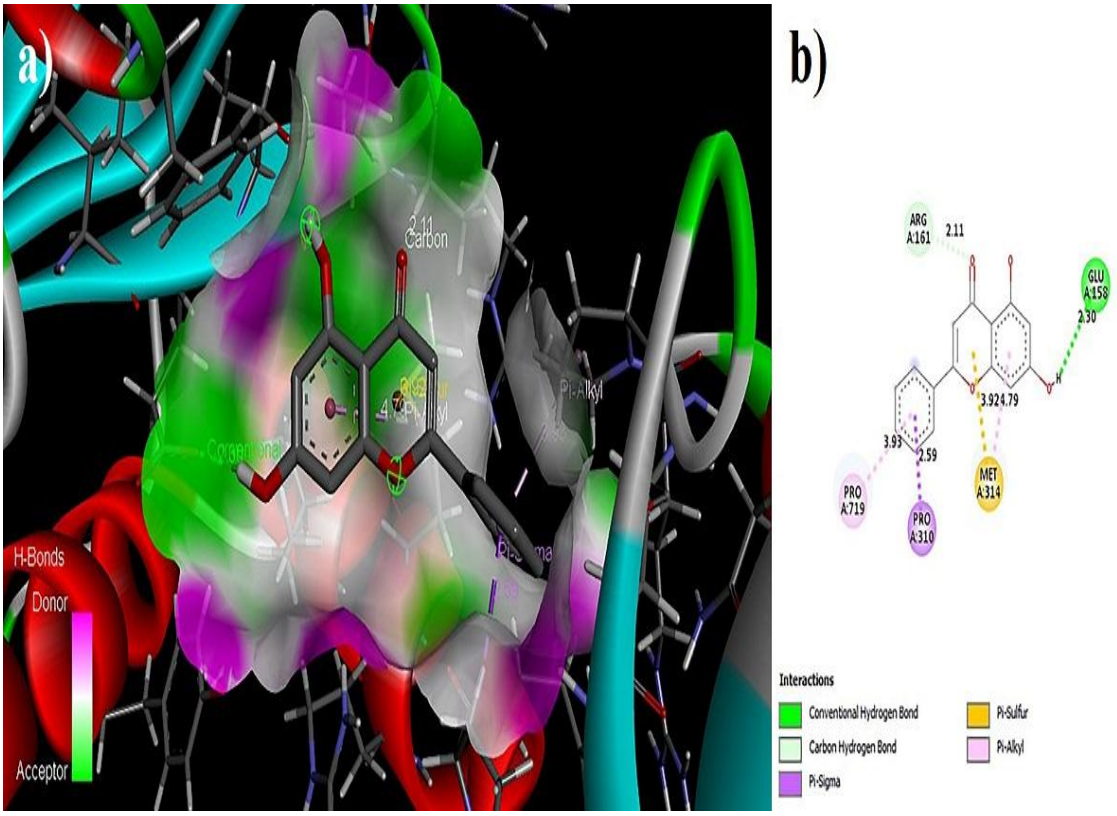

Figure 9: a) 3D structure representation of 5T93 protein-ligand complex, visualizing ligand binding within the hydrophobic pocket and highlighting molecular interactions. **b)** 2D interaction diagram of 1BM0 protein-ligand complex, showing conventional hydrogen bonds, Pi-Sulfur, Pi-Alkyl, and Pi-Sigma interactions contributing to ligand stability. 2D interaction diagram of 5T93 protein-ligand complex, depicting hydrogen bonding, Carbon Hydrogen Bonds, and Pi-Sulfur interactions stabilizing the ligand within the active site.

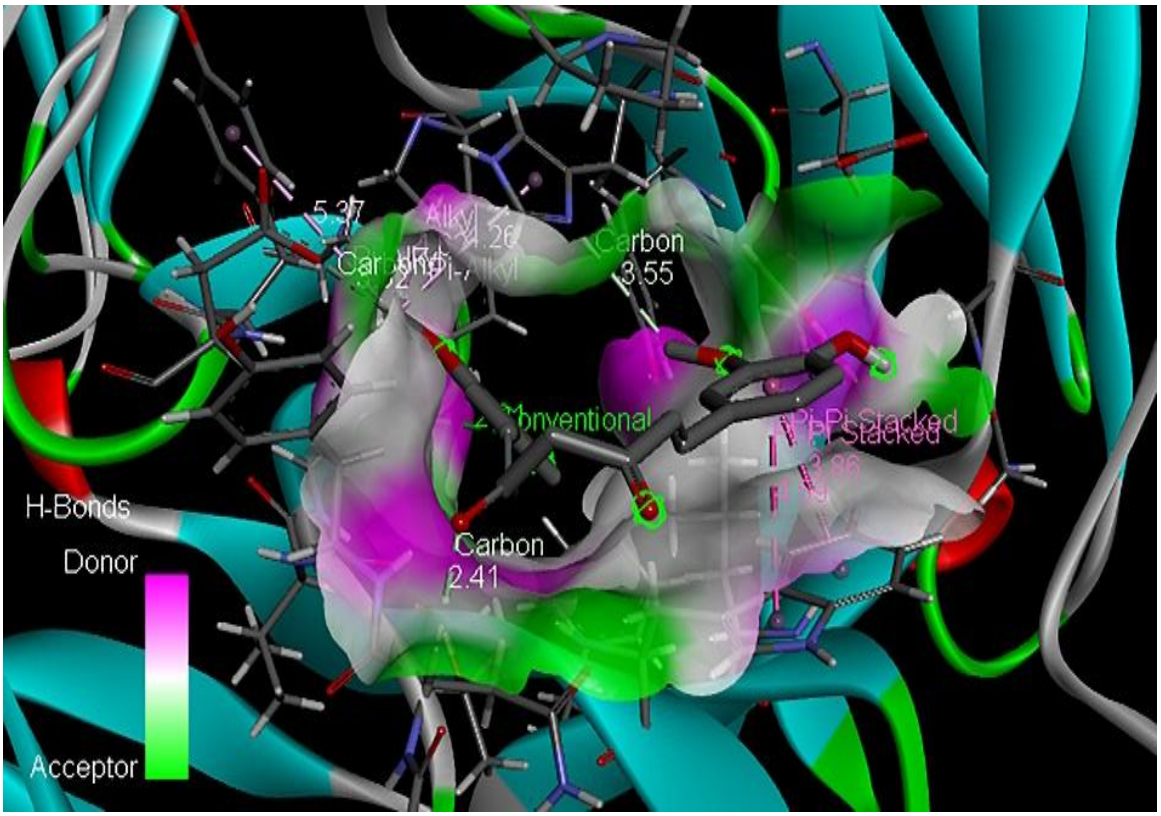

Figure. 10: 3D molecular docking representation showing the interaction of the 5T93 protein with the ligand. The hydrogen bond donor and acceptor regions are highlighted, along with various interaction types stabilizing the ligand within the binding pocket.

Table 1: Diet and Drugs Administration Plan in Albino Mouse during the Experimental Period.

Groups	Treatments
Group 1: Negative Group/Vehicle	Routine diet + normal saline (1.0 ml/ Kg body wt. orally)
Group 2: Positive Hepatotoxic control	Amikacin (35 mg/ Kg)
Group 3: Experimental group 1	Amikacin+ Curcumin (100 mg/ Kg)
Group 4: Experimental group 2	Amikacin+ Curcumin (200 mg/ Kg)
Group 5: Experimental group 3	Amikacin+ Curcumin (100 mg/ Kg) + honey (500 mg/ Kg)
Group 6: Experimental group 4	Amikacin+ Curcumin (200 mg/ Kg) + honey (500 mg/ Kg)

Experimental Groups 1 and 2 (different doses of *Curcuma longa*) show partial hepatoprotection and mild to moderate cell swelling. Experimental Groups 3 and 4 (Honey + *Curcuma longa*) show reduced toxicity, though the hepatocyte swelling is observed in some extent demonstrating dose-dependent protection.

3.3. Molecular Docking Analysis

The molecular docking study aimed to evaluate the interactions between hepatoprotective ligands and liver function-related enzymes using AutoDock Vina. The docking scores and interaction characteristics were analyzed to assess binding affinities and potential therapeutic effects.

3.3.1. Luteolin Binding Interactions with Bilirubin

The docking analysis of luteolin (CID: 5280445) with bilirubin UDP-glucuronosyltransferase (UGT1A1) – PDB ID: 2O6L revealed multiple molecular interactions, including hydrogen bonding and π-interactions. 3D docking visualization (Figure. 7a) displays the binding conformation of luteolin within the UGT1A1 active site. The molecular surface representation highlights key interaction regions, indicating strong binding affinity. 2D interaction mapping (Figure. 7b) illustrates specific interactions, including Conventional hydrogen bonds with GLN-459 (2.65 Å) and PRO-110 (2.46 Å) and Carbon hydrogen bonds with ARG-195 (1.37 Å). Pi-stacking interactions with HIS-146 and ARG-197, suggest additional stabilizing forces. Unfavorable donor-donor interaction with ARG-195 may impact binding efficiency. The presence of multiple hydrogen bonds and π-interactions suggests a stable ligand-protein complex, supporting luteolin's potential hepatoprotective role [32].

3.3.2. Quercetin Binding Interaction with Albumin

The molecular docking study was conducted to analyze the interactions between Quercetin (CID: 5280343) with Albumin (Serum albumin)– PDB ID: 1AO6. The goal was to assess binding affinity and potential hepatoprotective effects. Docking analysis revealed that hesperetin binds effectively within active site of PPAR-γ, engaging in multiple non-covalent interactions. 3D docking visualization (Figure 8a) illustrates binding pose of hesperetin in the active site of PPAR-γ, showing molecular surface and key interaction sites. 2D interaction mapping (Figure 8b) highlights the specific molecular interactions; Conventional hydrogen bonds with GLY-85 (2.57 Å), GLU-86 (2.62 Å), and TYR-84 (1.67 Å), suggesting strong stabilization, Carbon hydrogen bonds with HIS-105 (2.45 Å), GLN-32 (2.62 Å and 2.53 Å), and LEU-31 (2.64 Å), further enhancing binding affinity. Pi-sulfur interaction with MET-87 (4.35 Å), may contribute to ligand's stability within receptor pocket. These interactions suggest stable ligand-protein complex, reinforcing potential hepatoprotective activity of quercetin [33].

3.3.3. Docking Analysis of AST/Chrysin protein-ligand interaction

The molecular docking analysis of Chyrsin (CID:5281607) with AST (Aspartate Aminotransferase) PDB ID: 3K5M protein-ligand interaction revealed significant interactions stabilizing the ligand within the active site [34]. The 3D structure demonstrates the binding pocket's composition, highlighting hydrogen bond donors and acceptors contributing to ligand stabilization (Figure 9a). The 2D interaction diagram confirms various molecular interactions, including conventional hydrogen bonds with ARG A: 161 (2.11 Å) and GLU A:158 (2.30 Å), ensuring ligand anchoring within the binding cavity (Figure 9b). Additionally, Pi-Sulfur interactions with MET A:314 (3.92-4.79 Å) and Pi-Alkyl interactions involving PRO A:310 (2.59 Å) and PRO A:719 (3.93 Å) further enhance ligand-protein affinity. Pi-Sigma interactions are also observed with PRO A:310, contributing to overall stabilization of the complex.

3.3.4. Curcumin Binding Interactions with ALT

The molecular docking analysis of the Curcumin (CID: 969516) with ALT (Alanine aminotransferase) PDB ID: 5T93, a structural component involved in crucial biological functions, with the selected ligand, revealed significant interactions stabilizing the ligand within the binding pocket. In the 3D structure, the ligand was found to form multiple stabilizing interactions, including conventional hydrogen bonds, carbon-hydrogen bonds, and Pi-stacked interactions. The hydrogen bond donor and acceptor regions were visualized, demonstrating strong ligand-protein affinity. The presence of alkyl and Pi-alkyl interactions further contributed to the binding stability [35]. The 2D interaction diagram (not shown here) confirmed these findings by mapping the ligand's interaction with key amino acid residues. The ligand established conventional hydrogen bonding with SER B: 35(2.91A) but carbon-hydrogen bond with HIS D:39 and ASN B:32(2.41-4.26A), reinforcing its strong affinity for the protein. Additionally, Pi-alkyl interactions were noted with TYR B: 50 (5.37A) and PRO D:45 (4.62A), while Pi-stacked interactions with TRP B:92 (4.22A) further stabilized the complex. These interactions collectively suggest a high binding affinity of the ligand towards the 5T93 protein, making it a promising candidate for further biochemical studies (Figure 10).

3.3.5. Analysis of binding energies

The molecular binding score of the ligand/proteins was calculated. So, ALT Curcumin binding energy score was -7.6 Kcal/mol. AST with Chyrsin energy score was -7.5 Kcal/mol and Albumin, Quercetin binding energy score was -7.6 Kcal/mol. In the end, the bilirubin with leutoline binding score was 8.1 Kcal/mol.

4. Conclusions

The present study demonstrates that curcumin and honey possess strong hepatoprotective activity against amikacin-induced liver injury in a murine model. The combined therapy reduced elevated levels of liver enzymes significantly, normalized histopathological liver architecture, and exhibited strong molecular interactions with major liver enzymes. The biochemical and histological findings establish that curcumin and honey synergistically suppress oxidative stress and inflammation and thereby preserve hepatic function. These observations indicate the potential for therapeutic use of such natural products as adjuvants in drug-induced hepatotoxicity. Clinical studies are indicated to establish their efficacy and to explore therapeutic applications to human liver disease.

Conflict of Interest

All authors declare no conflict of interest.

Kindly Provide the Abbreviations

- ALT – Alanine Aminotransferase
- AST – Aspartate Aminotransferase
- ALB – Albumin
- TP – Total Protein
- TB – Total Bilirubin
- DILI – Drug-Induced Liver Injury
- ROS – Reactive Oxygen Species
- NBF – Neutral Buffered Formalin
- MDH – Malate Dehydrogenase
- LDH – Lactate Dehydrogenase
- TRIS – Tris(hydroxymethyl)aminomethane (Buffer)
- NADH – Nicotinamide Adenine Dinucleotide (Reduced Form)
- UGT1A1 – UDP-glucuronosyltransferase 1A1
- PDB – Protein Data Bank
- ADT – AutoDock Tools
- SPSS – Statistical Package for the Social Sciences
- SE – Standard Error
- ANOVA – Analysis of Variance
- PPAR-γ – Peroxisome Proliferator-Activated Receptor Gamma

CID – Compound Identification (PubChem)

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