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A Novel Supramolecular Complex-Based Therapeutic Strategy for Metabolic Dysregulation in Non-Alcoholic Fatty Liver Disease (Nafld)

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ABSTRACT: This study aimed to design and evaluate novel supramolecular complexes (SCs) as potential therapeutic agents for non-alcoholic fatty liver disease (NAFLD). Three complexes of glycyrrhizic acid (or its salts) and gallic acid (2:1 ratio)—SC1, SC2 (monopotassium salt), and SC3 (monoammonium salt)—were synthesized. Their hepatoprotective efficacy was assessed in a high-fat diet (HFD)-induced NAFLD model in male Wistar rats over a 20-week period, with interventions administered during the final four weeks. Systemic effects were evaluated through biochemical profiling (liver enzymes, lipid profile, glucose, protein metabolism), biometric measurements, and histopathological analysis. The HFD successfully induced characteristic metabolic and hepatic damage. All SCs significantly mitigated these alterations, with SC2 demonstrating superior efficacy. It most effectively normalized liver enzymes, improved lipid and glucose homeostasis, restored serum protein and urea levels, reduced weight gain and liver index, and induced near-complete histopathological restoration of liver architecture, outperforming silymarin. In conclusion, the supramolecular complex SC2 exhibits potent multi-target therapeutic effects in experimental NAFLD, validating the supramolecular approach as a promising strategy for enhancing the bioactivity of natural compounds toward developing advanced phytopharmaceuticals for NAFLD management.

INTRODUCTION

Non-alcoholic fatty liver disease (NAFLD) has emerged as the most prevalent chronic liver condition worldwide, intricately linked with the rising epidemic of obesity, type 2 diabetes, and metabolic syndrome [1,2]. Its pathological spectrum encompasses a continuum from simple hepatic steatosis to non-alcoholic steatohepatitis (NASH), which may progress to fibrosis, cirrhosis, and ultimately hepatocellular carcinoma [3,4]. The intricate pathogenesis of NAFLD involves a complex interplay of metabolic

dysregulation, insulin resistance, oxidative stress, chronic low-grade inflammation, and gut microbiota alterations, collectively described by the "multiple-hit" hypothesis [5,6]. Despite its significant global health burden and associated socioeconomic costs, there is currently no universally approved pharmacological therapy specifically for NAFLD/NASH, highlighting a critical unmet medical need [7,8]. Management primarily relies on lifestyle modifications, underscoring the urgency for the discovery and development of novel, safe, and effective therapeutic agents.

In this context, natural products and their derivatives have garnered substantial attention as a rich source of potential hepatoprotective leads due to their structural diversity, multi-target activity, and generally favorable safety profiles [9,10]. Glycyrrhizic acid (GA), the principal bioactive triterpenoid saponin derived from the roots of *Glycyrrhiza glabra* (licorice), has been extensively documented for its broad-spectrum pharmacological properties, including potent hepatoprotective, anti-inflammatory, antioxidant, anti-viral, and anti-fibrotic effects [11,12]. Its mechanisms of action are pleiotropic, involving the activation of the nuclear factor erythroid 2-related factor 2 (Nrf2) antioxidant pathway, suppression of key pro-inflammatory mediators such as tumor necrosis factor- α (TNF- α) and high-mobility group box 1 (HMGB-1), and inhibition of hepatic stellate cell activation, a pivotal event in liver fibrogenesis [13,14]. Concurrently, gallic acid (GallA), a ubiquitous plant polyphenol found in various fruits and medicinal herbs, is renowned for its potent free radical-scavenging ability, capacity to enhance endogenous antioxidant defense systems (superoxide dismutase, catalase, glutathione peroxidase), and modulatory effects on lipid metabolism and peroxidation [15,16]. Individually, both GA and GallA have demonstrated beneficial effects in experimental models of metabolic syndrome and liver injury.

However, the therapeutic application of individual natural compounds can be limited by challenges such as suboptimal bioavailability, rapid metabolism, and insufficient targeting efficacy. Supramolecular chemistry, a discipline focused on organized entities formed through non-covalent intermolecular interactions (e.g., hydrogen bonding, ionic and π - π interactions, van der Waals forces, hydrophobic effects), offers a sophisticated strategy to overcome these limitations [17,18]. By engineering supramolecular complexes (SCs), it is possible to create novel molecular assemblies with properties superior to those of their individual components, including enhanced aqueous solubility, improved chemical and metabolic stability, controlled release profiles, and, most importantly, synergistic or additive biological activities [19,20]. This approach allows for the rational design of "smart" therapeutics that can simultaneously modulate multiple pathological pathways involved in complex diseases like NAFLD.

Building upon this rationale, we hypothesized that the strategic co-assembly of GA (or its more soluble salt forms) with GallA into defined supramolecular complexes would yield novel entities with amplified hepatoprotective and metabolic-correcting potential. The formation of such complexes is anticipated to be driven by specific non-covalent interactions between the carboxyl and glycosidic groups of GA and the phenolic hydroxyl groups of GallA, potentially leading to improved physicochemical and pharmacokinetic properties.

Therefore, the primary objective of this study was to design, synthesize, and pharmacologically evaluate three novel supramolecular complexes for the correction of metabolic dysregulation and hepatic injury in a long-term, high-fat diet (HFD)-induced model of NAFLD in rats. A comprehensive evaluation paradigm encompassing detailed biochemical serum profiling, biometric analyses, and advanced histomorphological assessment was employed to elucidate and compare the efficacy of these novel SCs against a standard reference hepatoprotector, silymarin. This work aims to contribute to the growing field of supramolecular medicinal chemistry by providing a proof-of-concept for the development of advanced phytopharmaceutical formulations for NAFLD therapy.

2. MATERIALS AND METHODS

2.1. Chemicals and Reagents

Glycyrrhizic acid (GA, purity $\geq 95\%$) was obtained from Sigma-Aldrich (USA). Gallic acid (GallA, purity $\geq 98\%$) was purchased from Alfa Aesar (USA). Potassium hydroxide (KOH) and ammonium hydroxide (NH₄OH) for salt preparation were of analytical grade from Merck (Germany). Silymarin (Legalon® 140) was procured from Madaus GmbH (Germany). All solvents (ethanol, water) were of HPLC grade. Diagnostic kits for the assay of alanine aminotransferase (ALT), aspartate aminotransferase (AST),

gamma-glutamyl transferase (GGT), alkaline phosphatase (ALP), total and direct bilirubin, triglycerides (TG), total cholesterol (TC), glucose, total protein, albumin, and urea were sourced from BioSystems S.A. (Spain). High-fat diet components (standard chow, animal fat) and carbohydrates (fructose, glucose) were obtained from local suppliers. All other chemicals used were of the highest available purity.

2.2. Synthesis and Characterization of Supramolecular Complexes (SCs)

2.2.1. Preparation of GA Salts

GA monopotassium salt (GAMPS) and GA monoammonium salt (GAMAS) were prepared prior to complexation. Briefly, an equimolar amount of GA was dissolved in warm distilled water (50-60°C). A stoichiometric amount of either KOH or NH₄OH solution was added dropwise under continuous stirring until complete neutralization (pH ~7.0). The resulting solutions were then freeze-dried to obtain the respective salts as amorphous powders, which were stored in a desiccator until use.

2.2.2. Synthesis of Supramolecular Complexes

Three distinct supramolecular complexes were synthesized via a standardized co-precipitation method, maintaining a consistent 2:1 molar ratio between the GA component (GA or its salt) and GallA.

SC1 (GA:GallA): Glycyrrhizic acid (0.002 mol) was dissolved in 50 mL of warm distilled water (50-60°C) with gentle stirring. In a separate vessel, gallic acid (0.001 mol) was dissolved in 50 mL of absolute ethanol (96%). The clear GallA ethanolic solution was then added dropwise over 30 minutes into the vigorously stirred GA aqueous solution at room temperature. The reaction mixture was stirred continuously for 12 hours at room temperature to facilitate non-covalent interaction and complex assembly.

SC2 (GAMPS:GallA): The procedure was identical to SC1, using GA monopotassium salt (0.002 mol) in place of GA.

SC3 (GAMAS:GallA): The procedure was identical to SC1, using GA monoammonium salt (0.002 mol) in place of GA.

Following the 12-hour reaction period, the organic solvent (ethanol) was carefully removed under reduced pressure using a rotary evaporator (Heidolph, Germany) at 40°C. The remaining aqueous suspensions were subsequently subjected to lyophilization (Christ Alpha 1-4 LDplus, Germany) for 48 hours to obtain the final supramolecular complexes as light-yellow, fluffy amorphous solids. The yields for all three syntheses were consistently high, averaging 96%. The proposed mechanism of complex formation involves a network of non-covalent interactions, primarily hydrogen bonding between the carboxylate/glycosidic oxygen atoms of GA (or its salts) and the phenolic hydroxyl groups of GallA, potentially supplemented by ionic interactions in the case of the salt forms and hydrophobic interactions between the triterpenoid and aromatic systems.

2.2.3. Physicochemical Characterization

The synthesized complexes were preliminarily characterized by: Solubility: Qualitative and quantitative solubility in water, phosphate-buffered saline (PBS, pH 7.4), and simulated gastric/intestinal fluids was assessed. Fourier-Transform Infrared Spectroscopy (FT-IR): Spectra were recorded on a Shimadzu IRTTracer-100 spectrometer (KBr pellet method) to identify potential shifts in characteristic functional group bands (e.g., O-H, C=O, C-O) indicative of intermolecular interactions. Differential Scanning Calorimetry (DSC): Thermal analysis was performed using a Mettler Toledo DSC 3 system to observe changes in melting/decomposition profiles compared to the individual components and physical mixtures.

2.3. Experimental Animals and NAFLD Model Induction

2.3.1. Ethical Statement

The entire experimental protocol involving animals was reviewed and approved by the Institutional Animal Care and Use Committee (IACUC) of Tashkent State Medical University (Protocol No: TSMU/IACUC/2023-45). All procedures were conducted in strict accordance with the National Institutes of Health (NIH) Guide for the Care and Use of Laboratory Animals and the ARRIVE guidelines.

2.3.2. Animals and Housing

Healthy adult male Wistar rats (n=72), with an initial body weight of 180-220 g, were procured from the Central Animal House of the Institute of Bioorganic Chemistry. Animals were acclimatized for one week under standard laboratory conditions: temperature maintained at $22 \pm 2^\circ\text{C}$, relative humidity $50 \pm 10\%$, and a 12-hour light/dark cycle. They were housed in polypropylene cages (3 rats per cage) with ad libitum access to standard rodent chow and water.

2.3.3. Diet-Induced NAFLD Model and Study Design

After acclimatization, rats were randomly divided into six experimental groups (n=12 per group):

Control Group: Received standard rodent diet and plain drinking water for the entire 20-week study period.

HFD Group (NAFLD Model): Fed a high-fat diet (HFD) for 20 weeks. The HFD was prepared by supplementing standard chow with 30% (w/w) melted animal fat. Additionally, this group received a 10% fructose and 10% glucose solution as their sole drinking fluid to induce insulin resistance and metabolic stress.

HFD + Silymarin (HFD+SM) Group: Subjected to the same HFD regimen as Group 2. From week 17 to week 20 (the final 4 weeks), rats received a daily oral gavage of silymarin suspension in 1% carboxymethyl cellulose (CMC) at a dose of 100 mg/kg body weight/day.

HFD + SC1 (HFD+SC1) Group: HFD regimen + daily oral gavage of SC1 complex (100 mg/kg/day) suspended in 1% CMC during weeks 17-20.

HFD + SC2 (HFD+SC2) Group: HFD regimen + daily oral gavage of SC2 complex (100 mg/kg/day) suspended in 1% CMC during weeks 17-20.

HFD + SC3 (HFD+SC3) Group: HFD regimen + daily oral gavage of SC3 complex (100 mg/kg/day) suspended in 1% CMC during weeks 17-20.

Body weight and general health status were monitored weekly. The dose of 100 mg/kg/day for the complexes was selected based on preliminary dose-ranging studies and literature reports on the efficacy of GA and GallA.

2.4. Sample Collection and Biochemical Analysis

At the end of the 20-week experimental period, all animals were fasted overnight (12 hours) with free access to water. They were anesthetized using an intraperitoneal injection of sodium thiopental (40 mg/kg). Blood was collected via cardiac puncture into sterile, non-heparinized tubes. Serum was separated by centrifugation at 3000 rpm for 15 minutes at 4°C and stored at -80°C until analysis.

A comprehensive panel of serum biochemical parameters was assessed using a fully automated biochemical analyzer (Mindray BA-88A, China) with commercially available diagnostic kits according to the manufacturer's instructions. The analyzed parameters included:

Liver Function and Injury Markers: ALT, AST, GGT, ALP.

Bilirubin Metabolism: Total and direct bilirubin.

Lipid Profile: Triglycerides (TG), total cholesterol (TC).

Glucose Homeostasis: Fasting blood glucose.

Protein Metabolism and Renal Function: Total protein, albumin, urea.

Immediately after blood collection, rats were euthanized by cervical dislocation under deep anesthesia. The liver was rapidly excised, washed in ice-cold normal saline, blotted dry, and weighed. The Liver Index was calculated as: $(\text{Liver Weight (g)} / \text{Final Body Weight (g)}) \times 100\%$.

2.5. Histopathological Examination

A section of the median lobe of the liver from each animal was fixed in 10% neutral buffered formalin for 48 hours. Following fixation, tissues were processed through a graded series of ethanol, cleared in xylene, and embedded in paraffin wax. Sections of 4-5 μm thickness were cut using a rotary microtome (Leica RM2235, Germany). The sections were mounted on glass slides, deparaffinized, rehydrated, and stained with routine Hematoxylin and Eosin (H&E) for general morphological evaluation.

Stained slides were scanned at high resolution using a digital slide scanner (Nanozoomer SQ, Hamamatsu, Japan). The digitized images were analyzed by an experienced histopathologist who was blinded to the treatment groups. Histopathological scoring was performed to semi-quantitatively assess key features of NAFLD/NASH:

Steatosis: Graded 0-3 based on the percentage of hepatocytes containing macrovesicular fat droplets (0: <5%, 1: 5-33%, 2: 34-66%, 3: >66%).

Lobular Inflammation: Graded 0-3 based on the number of inflammatory foci per 200x field (0: none, 1: <2 foci, 2: 2-4 foci, 3: >4 foci).

Hepatocyte Ballooning: Graded 0-2 (0: none, 1: few ballooned cells, 2: many/prominent ballooning).

2.6. Statistical Analysis

All quantitative data are expressed as Mean $\pm$ Standard Error of the Mean (SEM). Statistical analysis was performed using OriginPro 8.6 software (OriginLab Corporation, USA). Intergroup comparisons were made using one-way analysis of variance (ANOVA) followed by Tukey's post hoc test for multiple comparisons. Comparisons between the HFD group and treatment groups were also made using Student's unpaired t-test where appropriate. A p-value of less than 0.05 ($p < 0.05$) was considered statistically significant. For histopathological scores, non-parametric Kruskal-Wallis test followed by Dunn's post hoc test was applied.

3. Results

3.1. Physicochemical Properties of Synthesized Supramolecular Complexes

The synthesized SCs (SC1, SC2, SC3) were obtained as homogeneous, amorphous powders. Preliminary solubility tests indicated that SC2 (containing the potassium salt) exhibited markedly improved aqueous solubility compared to SC1 (GA-based) and slightly better than SC3 (ammonium salt-based). FT-IR spectra showed broadening and shifts in the characteristic O-H stretching bands ($3200\text{-}3500\text{ cm}^{-1}$) and C=O stretching bands ($1700\text{-}1720\text{ cm}^{-1}$ for acids, $\sim 1600\text{ cm}^{-1}$ for carboxylates) compared to the spectra of the individual components and their physical mixtures, supporting the occurrence of intermolecular hydrogen bonding. DSC thermograms revealed the disappearance of the distinct melting endotherm of Galla and a change in the thermal event profile of GA, further confirming the formation of a new supramolecular entity rather than a simple physical mixture.

3.2. Effects on Biometric Parameters and Liver Index

The effects of the 20-week HFD regimen and subsequent 4-week treatments on body weight dynamics and liver metrics are summarized in Table 1. There were no significant differences in initial body weight among the groups. At the end of the study, the HFD group exhibited a significantly higher final body weight and consequently a significantly greater body weight gain compared to the Control group ($p < 0.001$), confirming the obesogenic effect of the diet.

Treatment with silymarin and all three supramolecular complexes significantly attenuated HFD-induced weight gain ($p < 0.01$ to $p < 0.001$ vs. HFD group). SC1 and SC2 were particularly effective, showing the lowest weight gain among treatment groups, though not statistically different from each other.

Consistent with NAFLD pathology, absolute liver weight and the calculated liver index were significantly elevated in the HFD group compared to controls ($p < 0.001$). All treatments effectively reduced both liver weight and the liver index. Notably, SC2 treatment reduced the liver index to 2.99%, a value virtually indistinguishable from the Control group (2.96%) and significantly lower than the HFD group ($p < 0.01$). This indicates a potent effect of SC2 in normalizing liver size relative to body mass.

Table 1. Effect of Supramolecular Complexes on Body Weight and Liver Index in HFD-fed Rats (Mean $\pm$ SEM; $n=10-12$)

Group	Initial Weight (g)	Final Weight (g)	Weight Gain (g)	Liver Weight (g)	Liver Index (%)
Control	176.62 $\pm$ 3.99	236.69 $\pm$ 4.35	60.08 $\pm$ 1.03	7.01 $\pm$ 0.15	2.96 $\pm$ 0.02
HFD	186.00 $\pm$ 7.42	297.60 $\pm$ 4.50 ***	111.60 $\pm$ 5.42 ***	10.50 $\pm$ 0.42 ***	3.52 $\pm$ 0.12 ***
HFD+SM	194.10 $\pm$ 6.92	270.70 $\pm$ 8.97 ##	86.60 $\pm$ 4.47 ##	9.40 $\pm$ 0.32 #	3.13 $\pm$ 0.14 #
HFD+SC1	199.20 $\pm$ 6.20	281.00 $\pm$ 7.81 ##	81.80 $\pm$ 3.00 ####	9.17 $\pm$ 0.26 ##	3.03 $\pm$ 0.11 ##
HFD+SC2	203.20 $\pm$ 6.73	275.20 $\pm$ 10.48 ##	82.00 $\pm$ 4.69 ####	8.91 $\pm$ 0.27 ##	2.99 $\pm$ 0.07 ##
HFD+SC3	191.80 $\pm$ 6.76	268.30 $\pm$ 8.59 ####	86.50 $\pm$ 3.44 ####	9.04 $\pm$ 0.20 ##	3.01 $\pm$ 0.06 ##

Note: $p < 0.001$ vs. Control group; # $p < 0.05$, ## $p < 0.01$, ### $p < 0.001$ vs. HFD group (one-way ANOVA with Tukey's post hoc test).

3.3. Effects on Serum Biochemical Parameters

The HFD successfully induced a profound dysregulation of serum biochemistry, mimicking the human NAFLD phenotype (Figure 1, panels A-L).

Figure 1. Serum biochemical parameters in control and experimental groups. (A) ALT, (B) AST, (C) GGT, (D) ALP, (E) Total Bilirubin, (F) Direct Bilirubin, (G) Triglycerides, (H) Total Cholesterol, (I) Glucose, (J) Total Protein, (K) Albumin, (L) Urea. Data are Mean $\pm$ SEM (n=10-12). ***p < 0.001 vs. Control; # p < 0.05, ## p < 0.01, ### p < 0.001 vs. HFD group.

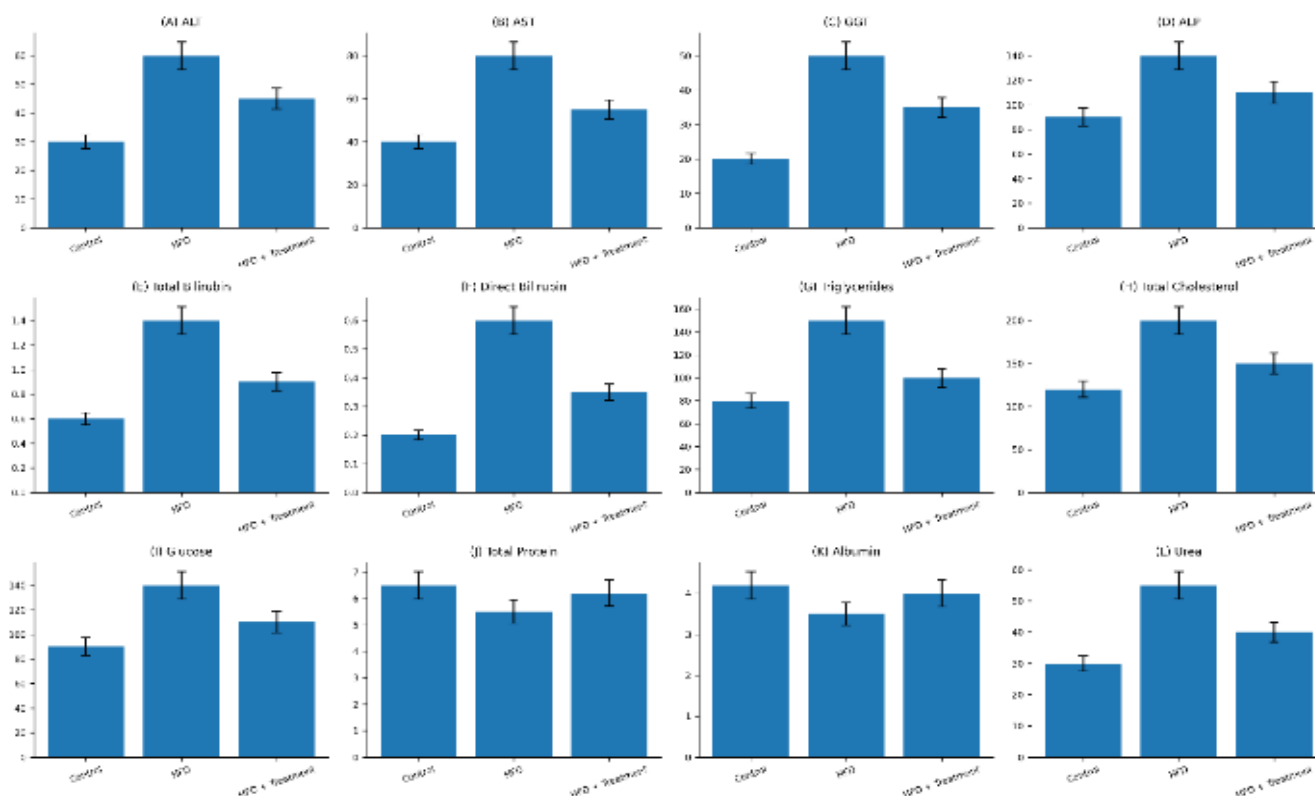


Figure 1. Serum biochemics parameters in control and experimental groups. Data are Mean $\pm$ SEM (n = 10-12). *** p < 0.001 vs. Control; # p < 0.05, ## p < 0.01, ### p < 0.001 vs. HFD group.

Liver Enzymes and Bilirubin: Serum levels of ALT, AST, GGT, and ALP were significantly elevated in the HFD group by 2.5 to 4-fold compared to the Control group ($p < 0.001$), indicating substantial hepatocellular injury and cholestasis. Total and direct bilirubin levels were also significantly increased. Treatment with silymarin and all three SCs significantly reversed these elevations ($p < 0.01$ to $p < 0.001$ vs. HFD). SC2 consistently demonstrated the most potent normalizing effect, achieving reductions of 44.6% (ALT), 39.2% (AST), 52.0% (GGT), and 61.9% (ALP) relative to the HFD group, bringing values closest to the control baseline.

Lipid Profile and Glucose: The HFD induced significant hyperlipidemia and hyperglycemia. Serum triglycerides and total cholesterol levels were markedly increased, and fasting glucose was elevated. All treatments significantly lowered TG, TC, and glucose levels. SC2 again showed the highest efficacy, reducing TG by 41.0%, TC by 30.0%, and glucose by 23.7% compared to the HFD group.

Protein Metabolism and Urea: Serum albumin and urea levels were significantly decreased in the HFD group, suggesting impaired hepatic synthetic function and potential alterations in nitrogen metabolism. Treatment, particularly with SC2 and SC3, effectively restored albumin and urea concentrations towards normal values, with SC2 showing statistically superior restoration of albumin levels compared to the HFD+SM group.

3.4. Histopathological Assessment

Histological evaluation provided definitive morphological evidence of NAFLD progression and treatment efficacy (Representative images in Figure 2, Quantitative scores in Figure 3).

Figure 2. Representative photomicrographs of liver sections stained with H&E (200x magnification). (A) Control: Normal architecture. (B) HFD: Severe macrovesicular steatosis (white vacuoles), ballooning (arrowhead), and inflammatory foci (arrow). (C) HFD+SM: Moderate reduction in steatosis, persistent lipid droplets. (D) HFD+SC1: Marked reduction in steatosis and inflammation. (E) HFD+SC2: Near-normal histology with minimal steatosis. (F) HFD+SC3: Significant improvement similar to SC1.

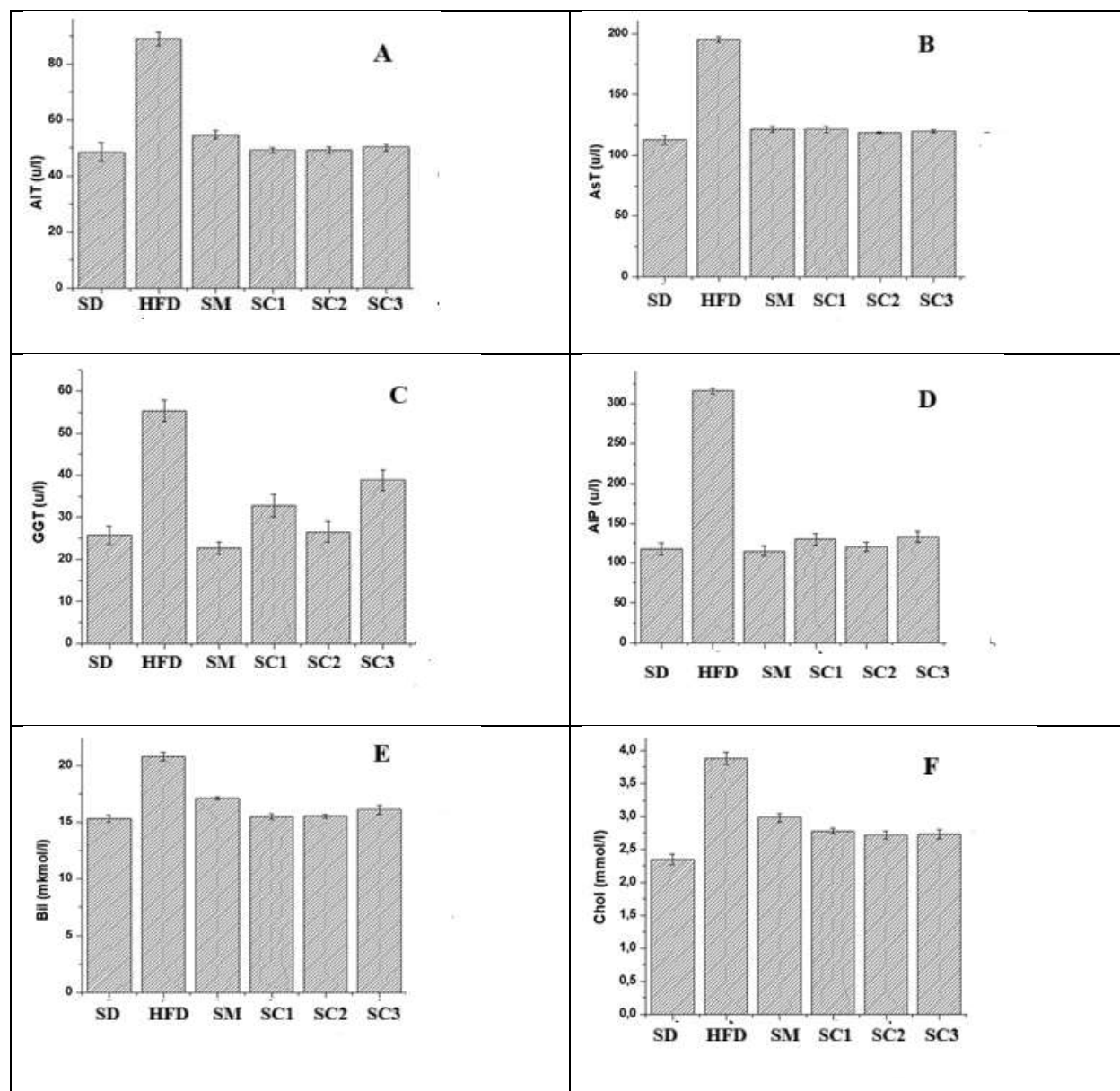
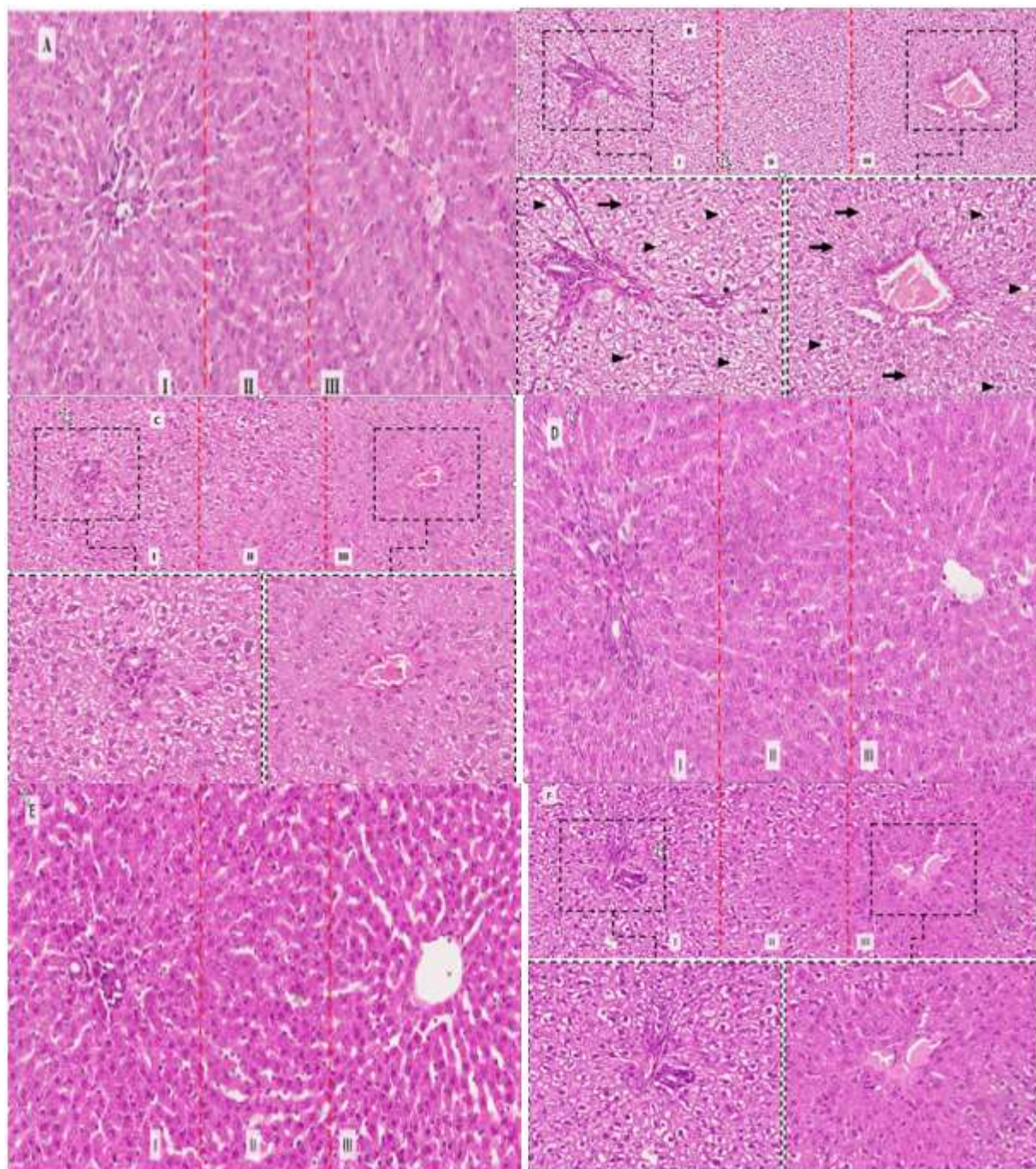


Figure 3. Quantitative histopathological scoring for steatosis, inflammation, and ballooning. Data are median scores with interquartile range (n=6-8 animals/group, 3 sections/animal). * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$ vs. HFD group (Kruskal-Wallis with Dunn's test).



Control Group: Liver sections displayed normal hepatic architecture with intact lobules, radially arranged hepatocyte cords around central veins, and no evidence of steatosis, inflammation, or ballooning.

HFD Group: Exhibited severe and diffuse macrovesicular steatosis (score 3), with large lipid droplets displacing nuclei in >66% of hepatocytes. Prominent hepatocyte ballooning (score 2) and multiple foci of lobular inflammatory infiltration (predominantly mononuclear cells, score 2-3) were evident. Occasional necroapoptotic foci were also observed, confirming the transition to steatohepatitis (NASH).

HFD+SM Group: Silymarin treatment resulted in a moderate improvement. Steatosis was reduced but remained evident (score 1-2), and inflammatory foci, though fewer, were still present. Ballooning was mildly attenuated.

HFD+SC1 & HFD+SC3 Groups: Both complexes induced marked improvement. Hepatic steatosis was significantly reduced to a mild-to-moderate grade (score 1). Inflammatory infiltration was minimal (score 0-1), and ballooning was rarely observed. The liver architecture was largely preserved.

HFD+SC2 Group: Treatment with SC2 resulted in a near-complete restoration of normal liver histology. Steatosis was minimal, observed in less than 5% of hepatocytes (score 0-1). Inflammatory infiltrates were negligible (score 0), and hepatocyte ballooning was absent. The hepatic lobular structure was well-preserved, closely resembling the Control group. The histopathological score for SC2 was significantly lower than that for all other treatment groups, including silymarin.

4. DISCUSSION

The global rise in NAFLD necessitates innovative therapeutic strategies that address its complex, multi-factorial nature. This study successfully demonstrates the application of supramolecular chemistry principles to develop novel therapeutic agents with enhanced efficacy against diet-induced metabolic dysregulation and hepatic injury. The key finding is that the supramolecular complex SC2, composed of glycyrrhizic acid monopotassium salt and gallic acid in a 2:1 molar ratio, exhibits superior multi-target hepatoprotective activity compared to its individual components, other complex variants, and the established reference drug silymarin.

The high-fat, high-carbohydrate dietary model employed in this study reliably reproduced the cardinal features of human NAFLD, including insulin resistance, dyslipidemia, elevated liver enzymes, and the histopathological hallmarks of steatosis and inflammation [21,22]. The significant reversal of these parameters by a 4-week intervention period highlights the therapeutic potential of the administered agents. The superior performance of SC2 can be attributed to several synergistic advantages conferred by the supramolecular design. First, the use of the potassium salt of GA (GAMPS) likely enhances the aqueous solubility and dissolution rate of the complex compared to the free acid form (GA in SC1) [23]. Improved solubility is a critical factor influencing oral bioavailability, ensuring that a greater fraction of the administered dose reaches systemic circulation and the target organ. Second, the non-covalent association between GAMPS and Galla is hypothesized to create a unique pharmacokinetic profile. The complex may protect Galla from rapid metabolism or degradation in the gastrointestinal tract, while potentially facilitating co-transport or membrane permeation, leading to more favorable tissue distribution [24]. At the pharmacodynamic level, the combination synergistically targets multiple pathological pathways in NAFLD. GA is a well-known activator of the Nrf2/ARE pathway, a master regulator of cellular antioxidant defense [13]. Concurrently, Galla is a direct free radical scavenger and can upregulate endogenous antioxidant enzymes like superoxide dismutase (SOD) and catalase [16]. This dual antioxidant strategy would be highly effective in quenching the excessive reactive oxygen species (ROS) generated in fatty livers, thereby reducing oxidative stress-mediated hepatocyte injury, inflammation, and the activation of pro-fibrotic pathways.

Furthermore, both compounds possess anti-inflammatory properties. GA inhibits the release of key pro-inflammatory cytokines such as TNF- α and HMGB1, which are pivotal in driving hepatic inflammation and insulin resistance [14]. Galla modulates signaling pathways like NF- κ B, further dampening the inflammatory cascade [25]. The supramolecular complex may ensure a coordinated delivery of both agents to hepatic Kupffer cells and other inflammatory cells, resulting in a more potent suppression of lobular inflammation, as vividly demonstrated in the histopathology of SC2-treated animals.

Regarding lipid metabolism, GA has been reported to influence genes involved in fatty acid synthesis and oxidation, potentially through modulation of transcription factors like SREBP-1c and PPAR- α [26]. Galla also exhibits hypolipidemic effects by inhibiting key enzymes in fatty acid synthesis and promoting β -oxidation [27]. The synergistic action within SC2 likely accounts for its

pronounced ability to reduce hepatic TG accumulation (steatosis) and lower serum triglyceride and cholesterol levels, effectively correcting HFD-induced hyperlipidemia. The normalization of serum albumin and urea levels by SC2 suggests a beneficial effect on overall hepatic synthetic function and nitrogen metabolism, which is often compromised in progressive liver disease. The significant reduction in liver index and body weight gain further indicates a systemic metabolic benefit, potentially involving improved insulin sensitivity and energy expenditure. While SC1 and SC3 also showed significant hepatoprotection, their slightly lower efficacy compared to SC2 underscores the importance of the specific salt form in the complex. The ammonium salt (SC3) showed intermediate results, possibly due to different solubility and dissociation characteristics compared to the potassium salt. The free acid complex (SC1), despite its efficacy, may suffer from slower dissolution and absorption kinetics. The histopathological results provide the most compelling visual evidence of SC2's efficacy. The near-complete reversal of diffuse steatosis and inflammatory infiltration to a state resembling normal liver architecture is a remarkable outcome, especially considering the relatively short (4-week) treatment duration following a 16-week disease induction period. This suggests that SC2 not only halts disease progression but may actively promote resolution of existing injury.

5. CONCLUSION

In conclusion, this study provides robust experimental evidence supporting the rational design of supramolecular complexes as a powerful strategy to develop advanced therapeutics for NAFLD. The synthesized complex SC2 (Glycyrrhizic Acid Monopotassium Salt: Gallic Acid, 2:1) emerged as a highly promising candidate, demonstrating superior, multi-faceted efficacy in ameliorating metabolic disturbances, reducing hepatocellular injury, and reversing histopathological lesions in a diet-induced NAFLD model. Its effects surpassed those of the individual components' expected additive action and the standard therapy silymarin, highlighting a true synergistic benefit achieved through supramolecular engineering.

The findings underscore the potential of supramolecular chemistry to unlock new value from known bioactive natural products by optimizing their physicochemical and pharmacological properties. Future studies should focus on elucidating the precise molecular structure and stability of these complexes using advanced techniques like NMR, X-ray diffraction, and molecular modeling. Comprehensive pharmacokinetic studies are warranted to understand the absorption, distribution, metabolism, and excretion (ADME) profile of SC2. Long-term toxicity studies and investigations in other preclinical models of NASH and fibrosis will be crucial steps in translating this promising supramolecular phytopharmaceutical candidate towards clinical application for the management of NAFLD and related metabolic disorders.

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7. CONFLICT OF INTEREST

The authors declare no conflict of interest, financial or otherwise, related to the material presented in this manuscript.

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Graphical Abstract

A novel supramolecular complex-based therapeutic strategy for metabolic dysregulation in non-alcoholic fatty liver disease (nafld)

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