

Assessment of the Ameliorative and Antihyperglycemic Effects of Fractions of *Ipomoea aquatica* in Male Rats with Streptozotocin-Induced Diabetes

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Abstract

This study aimed to investigate the potential ameliorative and antihyperglycemic effects of two fractions of *Ipomoea aquatica* on liver functions in streptozotocin (**STZ**)-induced diabetic male rats. Eighteen adult male albino rats were divided into three groups: control, **STZ** + 200 mg/kg **IA6-1** (dichloromethane-ethyl acetate fraction), and **STZ** + 200 mg/kg **IA9-2** (ethyl acetate-methanol fraction). *In vitro*, antiproliferative activity against liver cancer cell lines (HepG₂) was examined. Additionally, the plasma protein profile of **STZ**-induced diabetic rats was investigated by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (**SDS-PAGE**). Results showed a significant increase in serum alkaline phosphatase (**ALP**) and aspartate aminotransaminase (**AST**) in both treated groups compared to the control, whereas there was no significant difference between groups in the serum levels of alanine aminotransaminase (**ALT**), total protein, and albumin. The *in vivo* total antioxidant capacity (**TAC**) showed a significant increase in both treated groups compared to the control, indicating that *I. aquatica* may enhance the antioxidant defense system of the liver, which could help protect the liver from oxidative stress-induced damage. The profile of C-reactive protein (C-RP) (28.3 kDa) and apelin (16 kDa) in **STZ** rats showed a higher intensity compared to the control group and both treated groups. These proteins may play a role in regulating inflammation and oxidative stress in the liver, which are important mechanisms in the pathogenesis of liver diseases. Moreover, the **IA6-1** fraction had a more potent antiproliferative effect on liver cancer HepG₂ cell lines compared to the **IA9-2** fraction. This suggests that the plant may have a protective effect against liver damage caused by cancer cells. Overall, these findings highlight the potential therapeutic applications of *I. aquatica* for the treatment of liver diseases, particularly those associated with oxidative stress and inflammation. However, further research is needed to fully elucidate the mechanisms underlying the plant's beneficial effects on liver function and to determine optimal dosages and treatment durations for humans. Additionally, clinical trials will be necessary to confirm the safety and efficacy of *I. aquatica* as a therapeutic agent for liver diseases in humans.

Introduction

Liver diseases are a significant and growing public health issue that affects millions of people worldwide [1, 2]. Type 2 diabetes mellitus (**T2 DM**) is a prevalent metabolic disorder characterized by hyperglycemia and altered metabolism of lipids, carbohydrates, and proteins [3]. The liver plays a crucial role in detoxifying and eliminating xenobiotics and protecting the body against foreign substances [4]. However, **DM** is associated with several liver abnormalities, including abnormal glycogen deposition, non-alcoholic fatty liver disease (**NAFLD**), fibrosis, cirrhosis, hepatocellular carcinomas (**HCCs**), and viral hepatitis [5, 6]. Hyperglycemia-induced oxidative stress is one of the primary mechanisms underlying liver tissue injury in **DM**, leading to the derangement of protein, carbohydrate, and lipid metabolism, oxidative stress, and inflammation [7, 8].

The liver disease remains a serious health problem, and the evaluation of liver function is crucial in its diagnosis and management [9, 10]. Proteomics has emerged as a powerful tool for identifying differentially expressed proteins in health and disease tissue and has created opportunities for

investigating proteins as biomarkers [11, 12]. Apelin is a peptide that regulates fatty acid oxidation, adipogenesis, and insulin secretion in DM and plays an important role in the pathogenesis of **DM** complications [13].

Herbal medicines have attracted attention as an alternative cancer therapy due to their low cost and toxicity [14]. *I. aquatica* Forssk (Water Spinach) is an aquatic or semi-aquatic edible herb used traditionally for the treatment of liver malfunction, diabetes, constipation, arsenic, and heavy metal poisoning [15, 16]. The leaf extracts of *I. aquatica* have shown cytotoxic activity against **HepG₂** and A549 cell lines and a hepatoprotective effect against thioacetamide-induced liver cirrhosis [17]. Despite the various pharmacological properties exhibited by *I. aquatica* fractions and their potential applications in medicine, it is still underutilized due to a lack of knowledge and recommendations [18] and needs a full understanding of their mechanisms of action.

However, there have been some studies that have investigated the antidiabetic properties of *I. aquatica* extracts and fractions. Vijayakumar *et al.* [19] investigated the antidiabetic activity of various fractions of *I. aquatica* in streptozotocin-induced diabetic rats. The study found that the ethanolic extract of the plant exhibited significant antidiabetic effects, including a reduction in blood glucose levels and an increase in insulin secretion. However, the specific mechanisms underlying these effects were not elucidated. Yadav *et al.* [20] investigated the antidiabetic effects of an aqueous extract of *I. aquatica* in streptozotocin-induced diabetic rats. He found that the extract significantly reduced blood glucose levels and improved insulin sensitivity, possibly through the activation of the insulin signaling pathway and the inhibition of hepatic gluconeogenesis. Abu Bakar Sajak *et al.* [21] evaluated the effect of the green leafy vegetables of *I. aquatica* ethanolic extract using the ¹H **NMR** metabolomics approach on streptozotocin-induced diabetes rats. While these studies provide some insight into the anti-diabetic properties of *I. aquatica* extracts, more research is needed to fully understand the mechanisms by which specific fractions of the plant exert their ameliorative and antihyperglycemic effects in streptozotocin-induced diabetic rats.

In this study, we investigated the ameliorative and therapeutic effects of **IA6-1** and **IA9-2** fractions of *I. aquatica* on liver function. We assessed serum liver biomarkers, including **ALP**, **AST**, **ALT**, total protein, albumin, and plasma protein profile. Additionally, we examined the *in vivo* total antioxidant capacity and the *in vitro* anticancer activity of both fractions. Our study provides insights into the potential health benefits of *I. aquatica*, some insight into their mechanisms of action, and its potential use as a nutraceutical in the future.

Materials and methods

Chemicals and Apparatus:

Dimethyl sulfoxide (**DMSO**) and streptozotocin (**STZ**) were purchased from Sigma-Aldrich (St. Louis, USA), 3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (**MTT**) was obtained from Bio Basic Inc. (Markham, Canada) and **RPMI-1640** medium was purchased from Lonza Bioscience (Basel,

Switzerland). The biochemical tests included serum alanine aminotransferase (**ALT**), aspartate aminotransferase (**AST**), alkaline phosphatase (**ALP**), albumin, total protein, and total antioxidant capacity (**TAC**) obtained from a Biodiagnostic company (Giza, Egypt). The biochemical parameters and **TAC** were determined using a spectrophotometer UviLine 9400 (Mainz, Germany). Acrylamide, Bis-acrylamide, **SDS**, beta-mercaptoethanol, glycine, bromophenol blue, and Coomassie blue stains were purchased from Bio-Rad (Hercules, USA), and protein marker from Abcam (Cambridge, UK). Vertical gel electrophoresis and constant power supply were from Pharmacia (Uppsala, Sweden).

Plant material and sample preparation:

The aerial parts of *I. aquatica* were collected from Darfur (Sudan), dried at room temperature, and ground to obtain a fine powder. The extraction was carried out using maceration with methanol (100%) for 24 hrs. Later, the extract was filtered, and the solvent was removed using a rotary evaporator (40°C) to obtain a crude extract in a gummy form. The crude extract was then fractionated by vacuum liquid column chromatography on silica gel. The column was eluted with different solvent compositions, such as n-hexane, dichloromethane (**DCM**), ethyl acetate (**EtOAc**), methanol (**MeOH**), and finally with distilled water (100%), gradually increasing the polarity degree to obtain 15 fractions [22]. All fractions were evaporated to dryness using a rotary evaporator and stored at -20°C for further analyses.

Animal grouping and experimental design:

Adult male albino rats (210 ± 10 g) were obtained from the Animal House (Faculty of Science, Sohag University, Sohag, Egypt). Before experimentation, all animals were acclimated for a week. Furthermore, all animals were kept under environmentally controlled conditions of light (12-light/dark cycle, lights on at 8:00 a.m.) and temperature (24 ± 1°C) with free access to food and tap water. Finally, the animals were randomly divided into three groups (6 animals in each group). Group 1 was set as the normal control (**NC**) or non-diabetic group, which received 2% **DMSO** (v/v), while groups 2 and 3 were designated as diabetic groups and were treated by receiving orally the two *I. aquatica* fractions; **IA6-1** (**DCM:EtOAc**, 75:25, v/v) and **IA9-2** (**EtOAc:MeOH**, 50:50, v/v). The treatment was carried out at a dose of 200 mg/kg body weight (**BW**) for two weeks [23]. This work was executed under the guidelines of Sohag University and approved by the Ethics and the Regulation for Animal Care and Use Committee, Faculty of Medicine (Sohag, Egypt).

Induction of diabetes by STZ:

A single-dose intraperitoneal administration of a freshly prepared **STZ** solution (50 mg/kg **BW**) in ice-cold citrate buffer (0.1 M, pH 4.5) was used to induce diabetes [24]. 72 hours later, the blood glucose level was measured, and rats that revealed blood glucose levels over 250 mg/dL were accepted for the experiment.

Blood collection for Biochemical analysis:

After 15 days, all rats of each group were sacrificed, and blood samples for biochemical analysis were collected from the heart into plain tubes. Serum was separated by centrifugation at 3000 rpm for 10 min, and then transferred into Eppendorf tubes and stored at -20°C for further biochemical analyses.

Estimation of Biochemical Parameters:

The biochemical parameters in the serum of the control and treated groups were measured using different suitable kits. All assessment assays and kits were performed following the manufacturers' instructions and protocols.

Determination of Serum Total Protein and Albumin:

The serum total protein was performed using a Biuret colorimetric method with the serum protein kit according to a described methodology in [25]. The serum albumin was estimated using a bromocresol green (BCG) colorimetric method with a serum albumin kit following the manufacturer's instructions.

Assessment of Serum Liver Enzymes:

The serum alanine aminotransferase (**ALT**), aspartate aminotransferase (**AST**), and alkaline phosphatase (**ALP**) activities were determined using standard kits and following the manufacturer's instructions.

Assessment of Total Antioxidant Capacity (TAC - In vivo):

The **TAC** was measured using a commercial kit based on the ferric-reducing antioxidant power (**FRAP**) assay [26].

Plasma Protein Profiling:

The preparation of **SDS**-polyacrylamide gel electrophoresis was performed according to the method in [27]. The blood samples were collected from the control, **STZ**, T1, and T2 rat groups. The plasma was separated after centrifugation at 1000 rpm and dissolved in a sample buffer (62.5 mM Tris-HCl, pH 6.8, 25% glycerol, 2% **SDS**, 0.01% bromophenol blue, 5% β -mercaptoethanol). To denature the plasma proteins, 5 μ l of each plasma protein sample was mixed with 5 μ l sample buffer, and boiled at 95 °C for 5 min. Then, discontinuous **SDS-PAGE** on Tris-HCl 4 ~ 20% gel with a constant power of 200 V-h for 55 min. **SDS-PAGE** gels were stained with CBB, and the bands were quantified using Total Lab Quant. 2.2 for quantitative image analysis.

Western Blotting:

After **SDS-PAGE**, the separated proteins were transferred onto a nitrocellulose membrane using a Mini Trans-Blot® system (Bio-Rad, Hercules, CA, USA) according to the manufacturer's instructions. The membrane was blocked with 5% non-fat milk in Tris-buffered saline containing 0.05% Tween-20 (**TBST**) for 1 hour at room temperature.

A. Primary Antibody Incubation:

The membrane was incubated with primary antibodies against **C-RP** protein (1:500 dilution, Abcam, Cambridge, UK), **apelin** (1:1000 dilution, Santa Cruz Biotechnology, Santa Cruz, CA, USA), and actin (1:2000 dilution, Sigma-Aldrich, St. Louis, MO, USA) separately overnight at 4°C.

B. Secondary Antibody Incubation:

After washing with TBST, the membrane was incubated with secondary antibodies conjugated with horseradish peroxidase (HRP) (1:5000 dilution, Santa Cruz Biotechnology, Santa Cruz, CA, USA) for 1 hour at room temperature.

C. Chemiluminescent Detection:

The protein bands were visualized using a chemiluminescent substrate (SuperSignal™ West Pico PLUS, Thermo Fisher Scientific, Waltham, MA, USA) and imaged using a ChemiDoc™ Imaging System (Bio-Rad, Hercules, CA, USA).

D. Image Analysis:

The intensity of the protein bands was quantified using **ImageJ** software (National Institutes of Health, Bethesda, MD, USA) and normalized to the intensity of the actin band. The relative protein expression levels were compared between the **STZ**-induced rats and the control, **T1**, and **T2** groups.

Evaluation of Anticancer Activity (In-vitro):

A. Cell lines:

HepG2 cells, a human hepatocellular carcinoma cell line, were obtained from the American Type Culture Collection (ATCC, Rockville, MD). The cells were cultured in **RPMI** (1640) medium supplemented with 10% inactivated fetal calf serum and 50 µg/ml gentamycin. The cells were maintained at 37 °C in a humidified atmosphere with 5% **CO₂** and were sub-cultured two to three times a week [28].

B. Cell viability (MTT assay):

Cell viability was determined using the MTT assay to assess the cytotoxicity of *I. aquatica* fractions on **HepG₂** cells. **HepG₂** cells were seeded in 96-well plates at a density of 5×10^3 cells/well and incubated for 24 h. The cells were then treated with different concentrations of the *I. aquatica* fractions for 24 h. After incubation, **MTT** solution was added to each well and incubated for 4 h. The formazan crystals were dissolved in **DMSO**, and the absorbance was measured at 570 nm using a microplate reader [29]. The **IC₅₀** was estimated from graphic plots of the dose-response curve for each log concentration by non-linear regression analysis using GraphPad Prism software [30]. The percentage of cell viability was calculated from the following formula:

$$\text{Cell viability (\%)} = \frac{\text{The optical density of sample-treated cells (OD}_t\text{)}}{\text{The optical density of untreated-control cells (OD}_c\text{)}} \times 100$$

Statistical analysis:

All data were expressed as mean $\pm$ standard deviation (**SD**). Statistical analysis was performed using one-way analysis of variance (**ANOVA**) followed by Tukey's posthoc test using GraphPad Prism software version 8.0 [30].

The **ANOVA** test was used to determine whether there were significant differences among the means of the groups being compared, while Tukey's posthoc test was used to identify which specific groups were significantly different from each other. A p-value of less than 0.05 (*p < 0.05), less than 0.01 (**p < 0.01), or less than 0.001 (**p < 0.001) was considered statistically significant.

Results and Discussion

Biochemical Parameters:

Overall, the results of the biochemical assays revealed that fraction **IA6-1** (**DCM; EtOAc**) showed a more significant ameliorative effect on liver function parameters and total antioxidant capacity compared to fraction **IA9-2** (**EtOAc: MeOH**). These findings suggest that the bioactive compounds present in fraction **IA6-1** may have potential therapeutic applications in the treatment of liver diseases.

Serum Total Protein and Albumin:

The result of the serum total protein of the two fractions **IA6-1** and **IA9-2** exhibited a non-significant difference ($p > 0.5$) between both fractions and the control normal group as shown in **Table 1**. The results of the study suggest that the two fractions of IA, **IA6-1**, and **IA9-2**, have differential effects on liver function parameters and serum biomarkers. Fraction **IA6-1** was found to have a more beneficial effect on the production of albumin in the serum, while fraction **IA9-2** appeared to have a stimulating effect on the production of total protein. Both fractions were found to have an antioxidant effect, leading to an increase in the production of **TAC** in the serum. However, the albumin of the **IA9-2** treated group showed a close value than the fraction **IA6-1** (**Fig. 1**). As we observed, the results exhibited that both fractions **IA6-1** and **IA9-2** were found to restore the serum protein and albumin levels to the normal state.

Previous studies have shown that different fractions of plant extracts can have different chemical profiles and biological activities [31,32]. Abu Bakar Sajak et al. [21] detected changes in metabolites with 19 potential biomarkers due to *I. aquatica* ethanolic extract administration in obese diabetic-induced rats. Therefore, the bioactive compounds present in fractions **IA6-1** and **IA9-2** may have different mechanisms of action, leading to their differential effects on liver function parameters and serum biomarkers.

The observed antioxidant effect of both fractions is consistent with previous studies that have shown the hepatoprotective and antioxidant properties of plant extracts [33,34]. The antioxidant effect may be attributed to the presence of various bioactive compounds in the two fractions, including flavonoids, phenolic acids, and terpenes, which have been shown to possess antioxidant activity [35,36].

Serum Liver Enzymes:

As shown in **Table 2& Fig. 2**, The results show that the levels of **ALP** in both treatment groups, **IA6-1** and **IA9-2**, were significantly higher than the control group, with p-values of less than 0.001. The increase in **ALP** levels is an indicator of liver damage or injury, and the significant increase in **ALP** levels in both treatment groups suggests that the fractions may have a toxic effect on the liver [37,38].

Furthermore, the levels of **AST** in the **IA6-1** group were significantly higher than the control group, with a p-value less than 0.05, while the levels in the **IA9-2** group were significantly higher than both the control and **IA6-1** groups, with a p-value less than 0.01. Similarly, the levels of **ALT** in the **IA9-2** group were significantly higher than in the control group, with a p-value less than 0.05. The significant increase in **AST** and **ALT** levels in the treatment groups further supports the hypothesis that the fractions may have a toxic effect on the liver [39,40].

Overall, the results suggest that treatment with **IA6-1** and **IA9-2** may have a toxic effect on the liver, as evidenced by the significant increase in **ALP**, **AST**, and **ALT** levels. However, the exact mechanism underlying this toxic effect is unclear and requires further investigation.

Our findings indicate that the fraction **IA6-1** is more potential than the other fraction **IA9-2** through the period of administration. This is consistent with our previous study [23], which investigated the hepatoprotective properties of fraction **IA6-1**. The study found that **IA6-1** contains terpenoids and steroid phytochemicals, which may be responsible for the hepatoprotective property. These findings are in agreement with previous studies that have reported the hepatoprotective and antioxidant properties of terpenoids and steroids [41,42].

Total Antioxidant Capacity (TAC-In vivo):

Results of the in vivo total antioxidant capacity of both fractions **IA6-1** and **IA9-2** indicated a significant increase ($p < 0.05$) compared to the normal control group with **TAC** values of 1.33 ± 0.04 and 0.99 ± 0.06 mM/l for **IA6-1** and **IA9-2**, respectively (**Fig. 3**). The phytochemical constituent of the methanolic extract of *I. aquatica* was performed by standard approaches, and the results revealed the presence of substantial quantities of phenolics, flavonoids, saponins, terpenoids, and steroids. Subsequently, **UPLC-QTOF-MS** fingerprinting analysis of the fraction **IA9-2** recognized the presence of phenolics, such as salicylic acid and dihydroxybenzoic acid in, besides flavonoids, such as rutin, isoquercitrin, and nicotiflorin [22].

Despite, the presence of all these bioactive compounds in the fraction **IA9-2**, however, the fraction **IA6-1** has exhibited more potent **TAC**, which is probably due to synergistic effects resulting from the combination of flavonoids and other phenolics in the fraction **IA9-2**. The presence of sugar moiety in the compounds of fraction **IA9-2** may be attributed to its low activity, which is probably due to steric hindrance by the addition of sugar moieties [43].

Plasma Protein Profiling:

The standard protein curve of the migration distance (**Rf**) and log molecular weight protein markers (**Fig. 4**) showed a regression line with an equation $y = -0.1850 X + 128.4$ ($R^2 = 0.6956$), indicating acceptable accuracy [44]. Using the standard curve equation, the molecular weight range of the prestained protein marker was determined to be between **17-180 kDa**. The protein composition analysis by molecular weight (**Fig. 5**) showed that all plasma samples, including control, **STZ**-induced hyperglycemic, **T1**, and **T2**, had similar protein composition, including **ankyrin** (200 kDa), **IgG** (150 kDa), **nephline** (136 kDa), **IDE** (112 kDa), **albumin** (65 kDa), **prealbumin** (55 kDa), **CICP** (43 kDa), **ApoA-V** (39 kDa), **GAPDH** (35 kDa), **C-RP** (28.3 kDa), **leptin** (17 kDa), and **apelin** (16 kDa). The protein composition observed in this study is consistent with the protein types found in normal blood plasma, where **albumin** and **globulin** ($\alpha 1$, $\alpha 2$, β , and γ) are the major components [45].

The present study also demonstrated a significant positive association between **C-RP** and **apelin** protein levels in hyperglycemic animals induced by **STZ**. **Apelin** is a peptide hormone that plays a key role in regulating energy balance and glucose homeostasis [46]. The significant increase in **C-RP** and **apelin** protein expression observed in hyperglycemic animals compared to control suggests that inflammation may be associated with the development of diabetes and its complications. Previous research has established that **C-RP** levels are higher in people with diabetes [47], and the present study further supports this finding. The intensity of protein bands detected by western blotting (**Figs. 6 & 7**) is proportional to the amount of protein present in the sample [48,49], suggesting that the increased intensity of **C-RP** and **apelin** bands observed in **STZ**-induced rats may be due to higher protein concentrations.

The results of the study show that there is a significant increase in the expression of both **apelin** and **C-RP** in the **STZ**-induced rats compared to the **control** group. The **apelin/actin** ratio (**Fig. 7**) is significantly higher in the **STZ**-induced rats and **T1** group compared to the control group, with a 22.21-fold increase in the **STZ**-induced rats and lowered to be 12.33-fold in the **T1** group. Similarly, the **C-RP/actin** ratio is significantly higher in the **STZ**-induced rats and **T1** group compared to the control group, with a 25.49-fold increase in the **STZ**-induced rats and lowered to be 10.62-fold in the **T1** group.

While, the increase in the expression of **apelin** and **C-RP** in the **STZ**-induced rats is consistent with previous studies that have reported an increase in these proteins in diabetes and related complications [50,51], the increase in **apelin** expression in the **STZ**-induced group may reflect an adaptive response to the metabolic stress induced by diabetes.

The presence of **apelin** in hyperglycemic plasma suggests metabolic disorders, such as insulin resistance and increased energy production through glycolysis and gluconeogenesis [52,53]. Hyperglycemia alters **Akt/PKB** activation and induces insulin resistance [54,55]. Inhibition of protein kinase **B/Akt** activity interferes with the action of **GLUT4** transporters to transport glucose into cells [56].

TNF- α , an inflammatory **cytokine** elevated during insulin resistance associated with obesity, increases **apelin** expression in human and mouse adipocytes [57]. In this regard, the expression of the **apelin** gene

in adipose tissue is increased by insulin and **TNF- α** . Therefore, the role of **apelin** in the regulation of energy and obesity-related disorders should be given attention to the pathophysiology of these conditions.

Anticancer Activity (In-vitro):

The results presented in **Table 3**, and **Fig. 8**, show the in vitro anticancer activity of two compounds, **IA6-1** and **IA9-2**, using the **MTT** assay. The **MTT** assay is a commonly used method for measuring cell viability and proliferation, which is based on the ability of viable cells to reduce a yellow tetrazolium salt to a purple formazan product [29].

The IC_{50} values of the two fractions **IA6-1** and **IA9-2** were determined for three different groups, including the control, **T1**, and **T2**. The IC_{50} value represents the concentration of the compound required to inhibit cell growth or viability by 50% [58].

The results showed that both **IA6-1** and **IA9-2** fractions have differential anticancer activity against the **HepG₂** cell lines. Among the two fractions, **IA9-2** demonstrated the most potent anticancer activity with an IC_{50} value of 984.67 ± 10.83 μ g/ml, while **IA6-1** exhibited low cytotoxicity with an IC_{50} value of 4259 ± 6.66 μ g/ml (**Fig. 8**). The anticancer activity of Doxorubicin was relatively low, with IC_{50} values of 107.98 ± 5.38 μ g/ml. The growth of all cells decreased with increasing concentrations compared to the **control**. The differential activity of the compounds against different cell lines may be attributed to differences in the genetic and molecular characteristics of these cells, including differences in the expression of drug targets, drug transporters, and/or drug-metabolizing enzymes [59]. The observed cytotoxic effects of the **IA6-1** and **IA9-2** fractions suggest that these fractions contain bioactive compounds that may be useful in the development of anticancer agents. The presence of flavonoids and phenolics in fraction **IA9-2** may contribute to its anticancer activity, as these compounds have been shown to have potent anticancer effects [60,61]. It is worth noting that the cytotoxicity of the **IA9-2** fraction was found to be concentration-dependent, which suggests that the observed anticancer activity may be mediated by the accumulation of the active compounds in the cells over time. This finding is in agreement with previous studies that have reported concentration-dependent cytotoxicity of natural compounds against cancer cells [62,63].

Further investigations such as molecular docking studies can be conducted to predict the binding affinities and interactions of the active components of the plant with the target proteins involved in diabetes. Thus, potential drug candidates can be identified and their mechanisms of action can be better understood. This information can provide valuable insight into the molecular mechanisms underlying the ameliorative and antidiabetic effects of *I. aquatica*, ultimately contributing to the development of new therapeutic agents for the management of diabetes and related metabolic disorders.

Conclusions

In this study, it was found that fraction **IA6-1** may have a positive impact on the production of **albumin** in the serum, while fraction **IA9-2** may stimulate the production of total protein. Both fractions also exhibited an antioxidant effect and led to an increase in the production of **TAC** in the serum.

The study also demonstrated significant changes in **C-RP** expression in hyperglycemic animals induced by **STZ**, suggesting that inflammation may be associated with the development of diabetes and persistent levels of hyperglycemia, while **aplein** expression reflect an adaptive response to the metabolic stress induced by diabetes and its involvement in involved in the production of energy by glycogenolysis and gluconeogenesis. Overall, the results of this study provide insights into the molecular mechanisms underlying the pathogenesis of diabetes and related complications.

The current study represents the first attempt to explore the potential benefits of the fractions derived from the plant. The biochemical findings indicated that both fractions ameliorated liver functions in diabetic-treated rats, with a greater improvement observed in the diabetic-treated group that received the fraction containing terpenoids compared to the fraction containing flavonoid glycosides. Additionally, the results suggested that *I. aquatica* contributes to having anti-hyperglycemic activity in **STZ**-induced diabetic rats. Fraction **IA9-2** also showed potential as a potent anticancer agent in reducing the viability of **HepG₂** cells in a concentration-dependent manner. Future research should focus on characterizing the bioactive compounds of the **IA6-1** fraction and investigating the toxicity of the **IA9-2** fraction toward different cancer cell lines.

Declarations

Authors' contributions: M.H.G., N.M.E., and T.N.H. prepared Figures 1-8, wrote and revised the main manuscript text. N.M.E., M.H.G., A.S., A.M.M., and T.N.H. carried out the experimental work. N.M.E., M.H.G., and T.N.H. participated in data interpretation. M.H.G., A.S., and M.A.A. conducted data analysis and statistical analysis. All authors read and approved the final version of the manuscript.

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Data availability: All relevant data are within the paper.

Consent for publication: Not applicable.

Ethical approval and consent: were obtained in accordance with the guidelines of Sohag University and were approved by the Ethics and the Regulation for Animal Care and Use Committee of the Faculty of Medicine (Sohag, Egypt) to ensure the humane treatment of all animals involved in the study.

Conflict of interest: The authors declare that there are no conflicts of interest.

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Tables

Table 1: presents the results of measuring the levels of serum total protein, albumin, and total antioxidant capacity (TAC) in the normal control group, as well as in the groups treated with **IA6-1** and **IA9-2**.

Group	Control	T-1	T-2
Parameter		(IA6-1)	(IA9-2)
Total protein (g/dl)	7.097 ± 0.2	6.745 ± 0.6 ^{ns}	7.737 ± 0.64 ^{ns}
Albumin (g/dl)	5.1 ± 0.56	6.748 ± 0.37 ^{ns}	5.379 ± 0.49 ^{ns}
TAC (mM/l)	0.745 ± 0.09	1.133 ± 0.04 [*]	0.99 ± 0.06 [*]

Results are expressed as Mean ± SEM, with six samples in each group. The notation "ns" indicates no significant difference observed (p > 0.05), while "*" indicates a significant difference at the level of p < 0.05. The statistical significance was determined using One-way ANOVA followed by the Newman-Keuls test.

Table 2: displays the levels of liver enzymes (ALP, AST, and ALT) in the normal control group, as well as in the groups treated with **IA6-1** and **IA9-2**.

Group	Control	T-1	T-2
Parameter		(IA6-1)	(IA9-2)
ALP (IU/L)	448.7 ± 10.6	628 ± 1.1 ^{***}	635.6 ± 1.3 ^{***}
AST (U/L)	23.88 ± 2.8	35.58 ± 2.4 [*]	42.2 ± 2.5 ^{**}
ALT (U/L)	5.85 ± 1.49	6.48 ± 1.86 ^{ns}	9.6 ± 1.6 ^{ns}

Results are expressed as Mean ± SEM, with six samples in each group. The notation "ns" indicates that there was no significant difference observed (p > 0.05), while "*" denotes a significant difference at the level of p < 0.05. Moreover, "**" and "***" indicate a significant difference at the levels of p < 0.01 and p <

0.001, respectively. The statistical analysis was conducted using One-way ANOVA followed by the Newman-Keuls test.

Table 3: presents the results of the *in vitro* MTT assay evaluating the anticancer activity of **IA6-1** and **IA9-2**.

Cell line	IC ₅₀ (µg/ml)		
	(M ± SEM)		
	DOX	T-1 (IA6-1)	T-2 (IA9-2)
HepG2	107.98 ± 5.38	4259.00 ± 6.66	984.67 ± 10.83

Figures

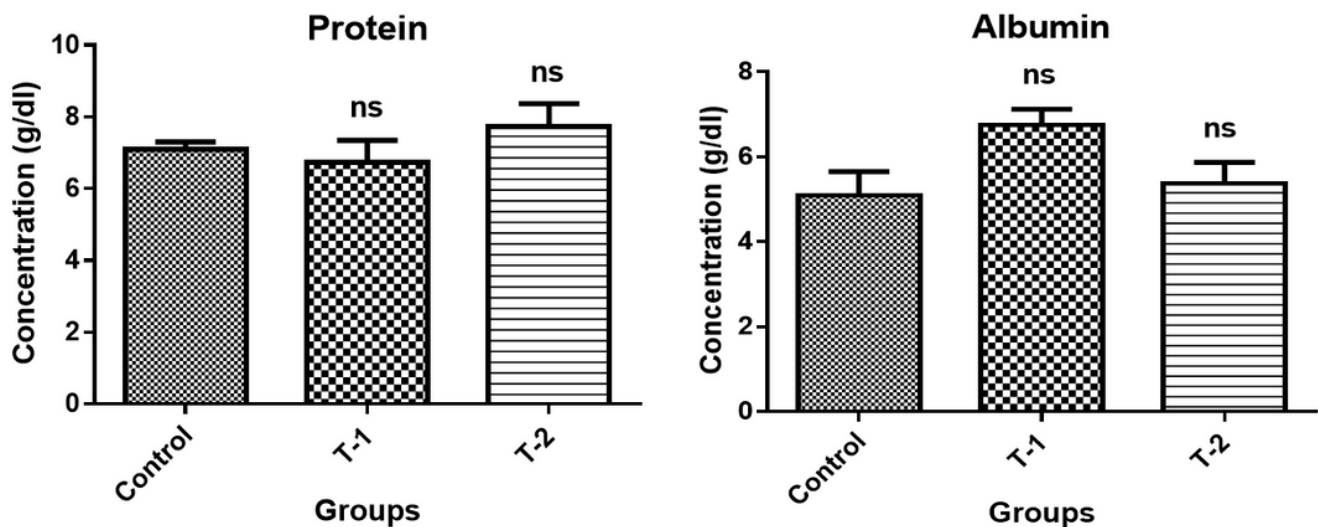


Figure 1

displays the influence of two fractions of *I. aquatica*, **IA6-1**, and **IA9-2**, on the levels of serum total protein and albumin. The data are presented as Mean ± SEM, with six samples in each group. The notation "ns" indicates no significant difference ($p > 0.05$), while "*" denotes a significant difference at the level of $p < 0.05$. The statistical significance was determined using One-way ANOVA followed by the Newman-Keuls test.

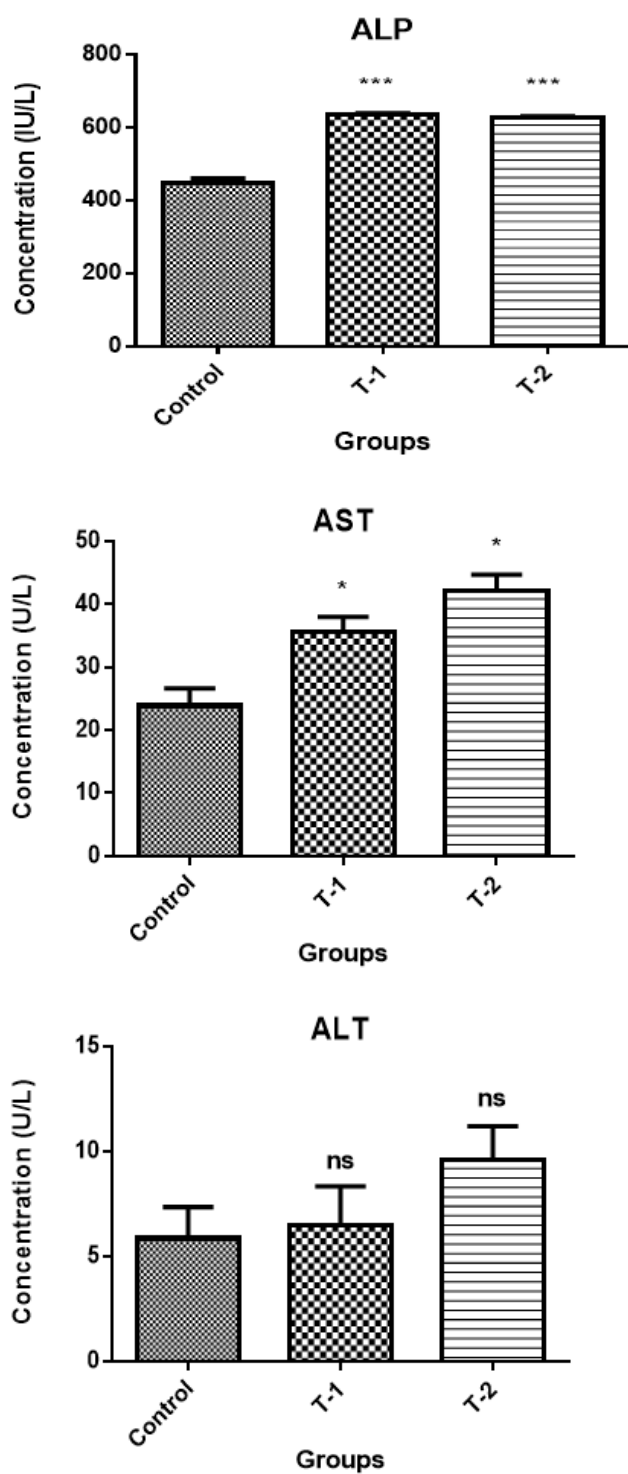


Figure 2

illustrates the impact of two fractions of *I. aquatica*, namely **IA6-1** and **IA9-2**, on the levels of liver enzymes (**ALP**, **AST**, and **ALT**). The data are presented as Mean $\pm$ SEM, with six samples in each group. The notation "ns" indicates no significant difference ($p > 0.05$), while "*" denotes a significant difference at the following levels: * ($p < 0.05$), ** ($p < 0.01$), and *** ($p < 0.001$). The statistical significance was determined using One-way ANOVA followed by the Newman-Keuls test.

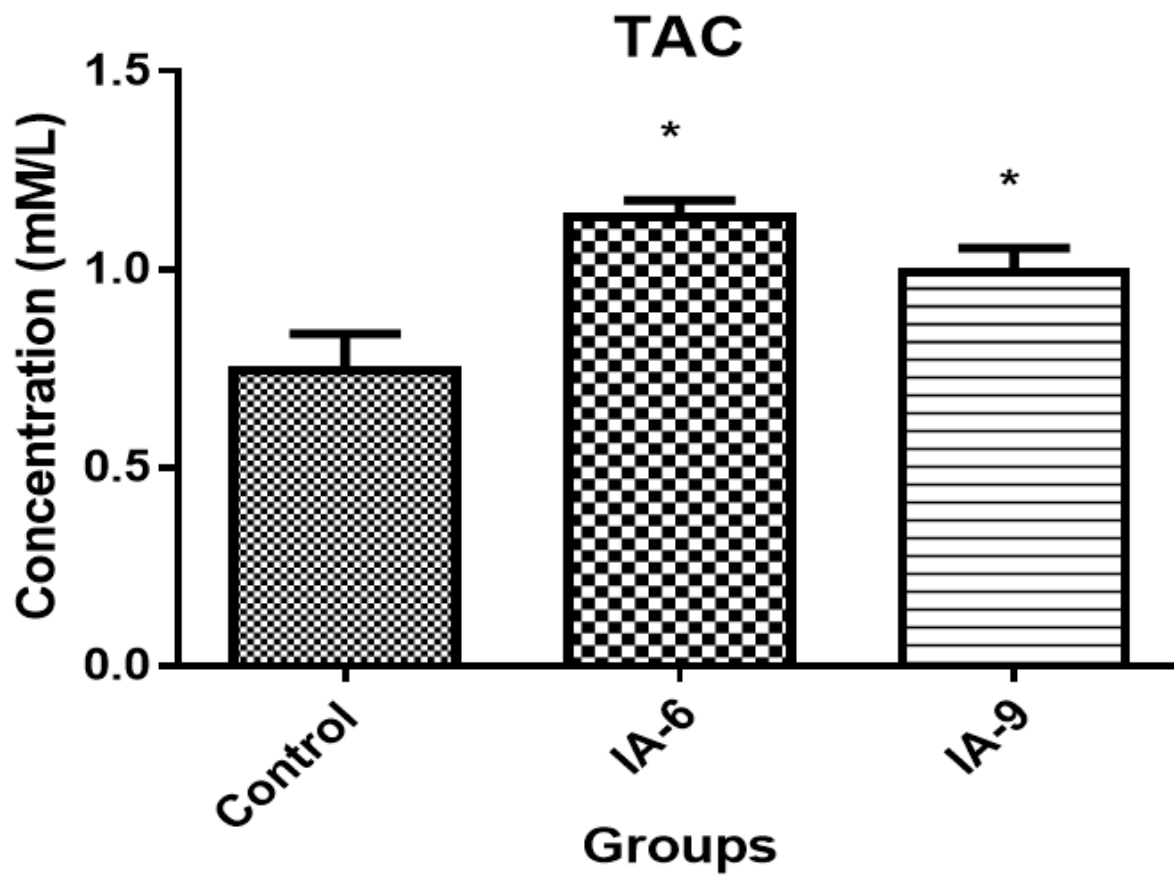
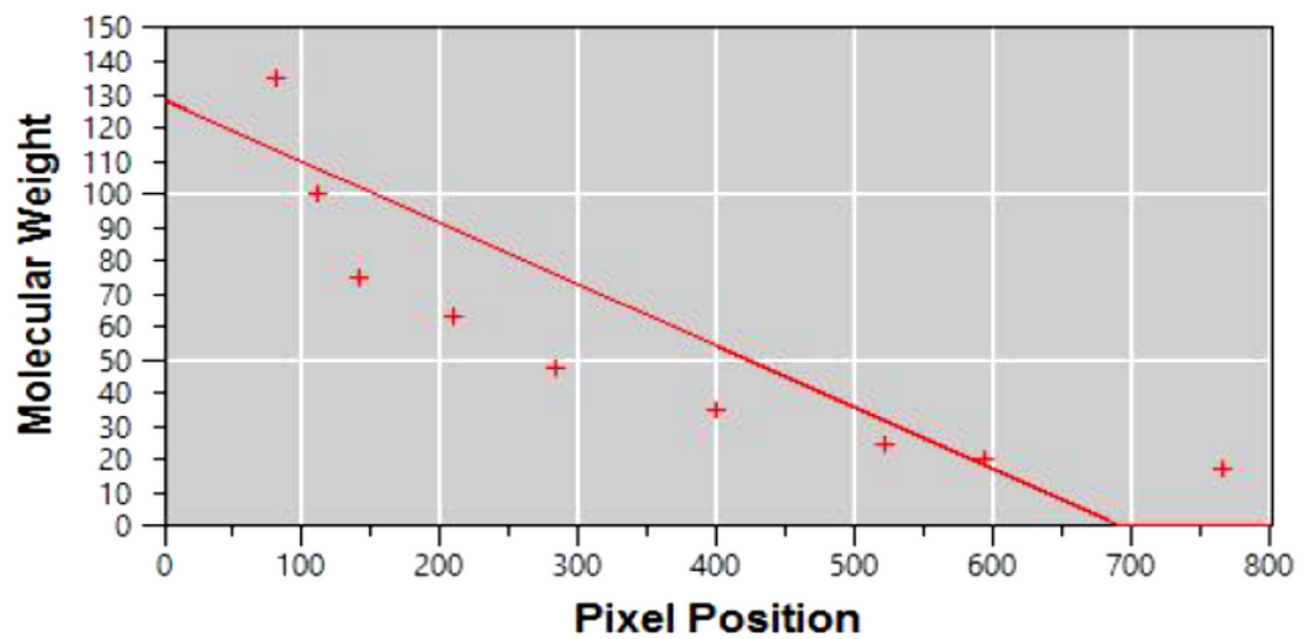


Figure 3

shows the *in vivo* total antioxidant activity of the two fractions, **IA6-1** and **IA9-2**.



Linear fit
 $y = -0.1850x + 128.4$
 R^2 Value: 0.6956

Figure 4

depicts the standard curve of the protein marker, with the y-axis representing the logarithm of the molecular weight (**MW**) of the protein and the x-axis representing the migration distance (**Rf**).

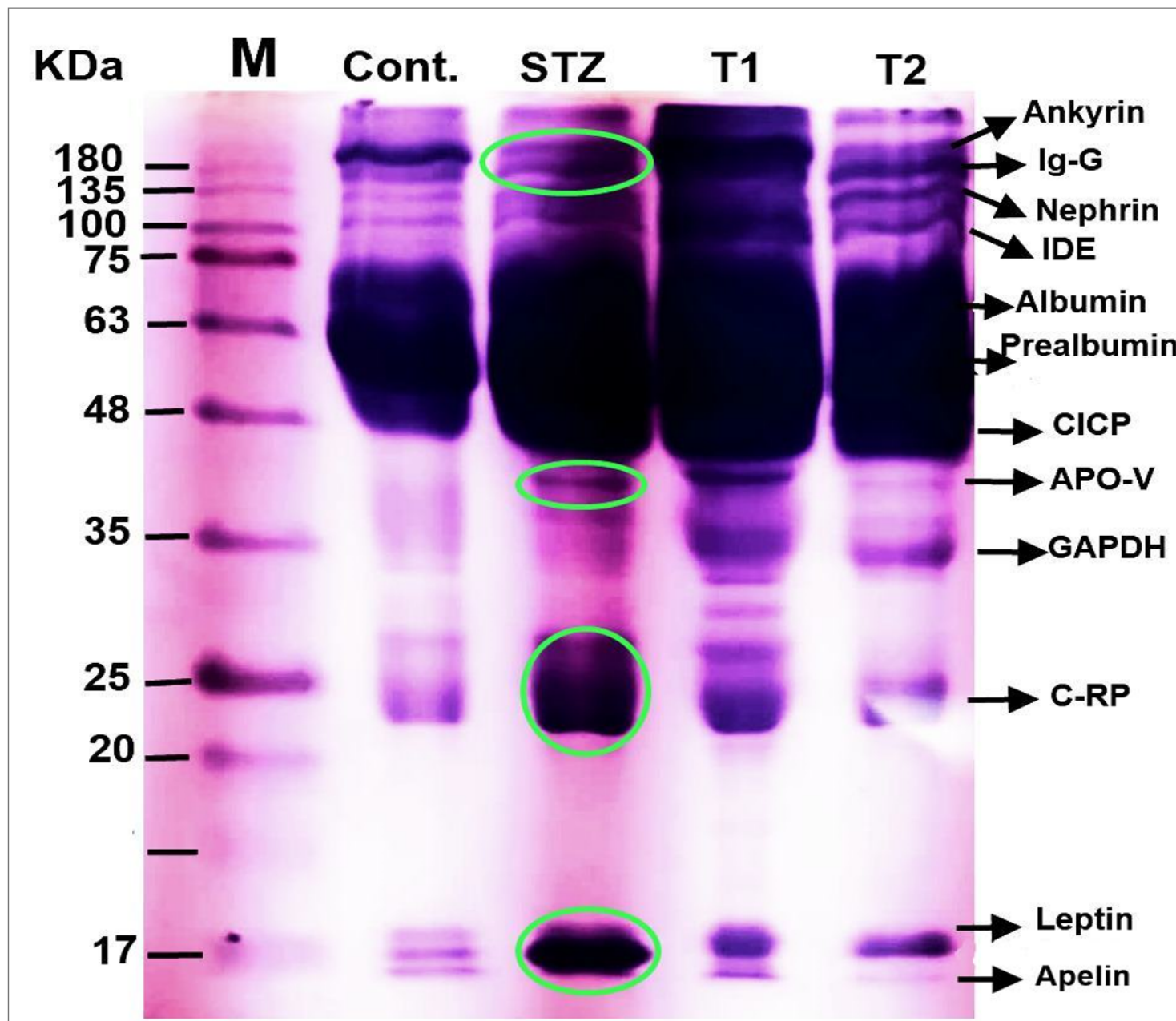


Figure 5

depicts a photomicrograph displaying the migration pattern bands of rat protein blood plasma after undergoing **SDS**-gel electrophoresis. The bands are labeled as follows: M: the protein marker, Cont.: the vehicle control, **STZ**: the streptozotocin treatment group, T1: the treatment group with fraction **IA6-1**, T2: the treatment group with fraction **IA9-2**.

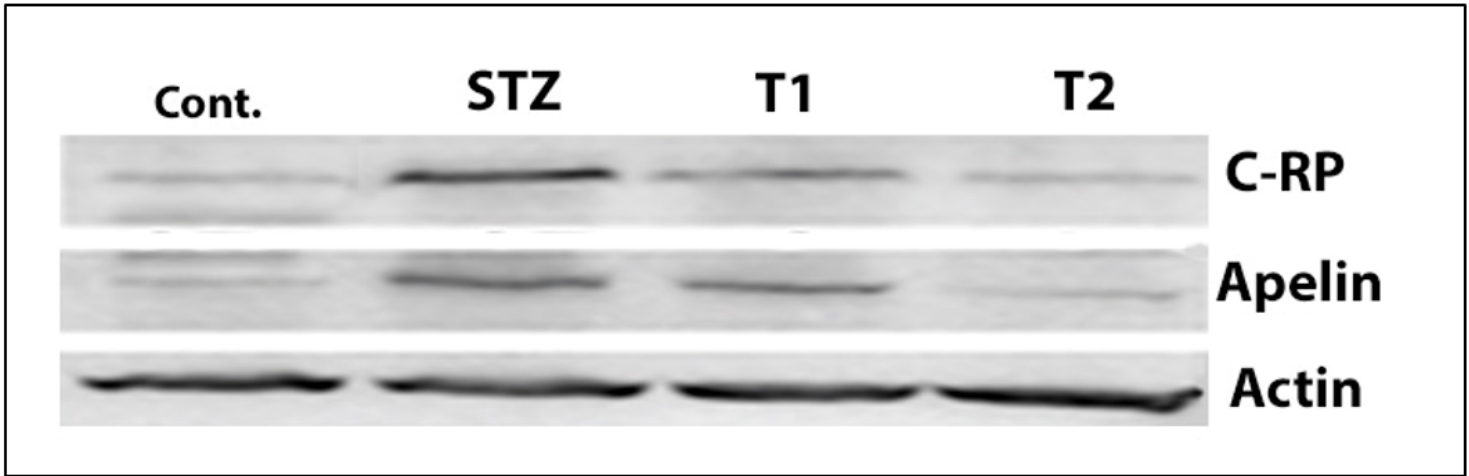


Figure 6

Western blot analysis revealed higher intensities of **C-RP** and **apelin** protein bands in **STZ**-induced rats compared to the Control, **T1**, and **T2** groups.

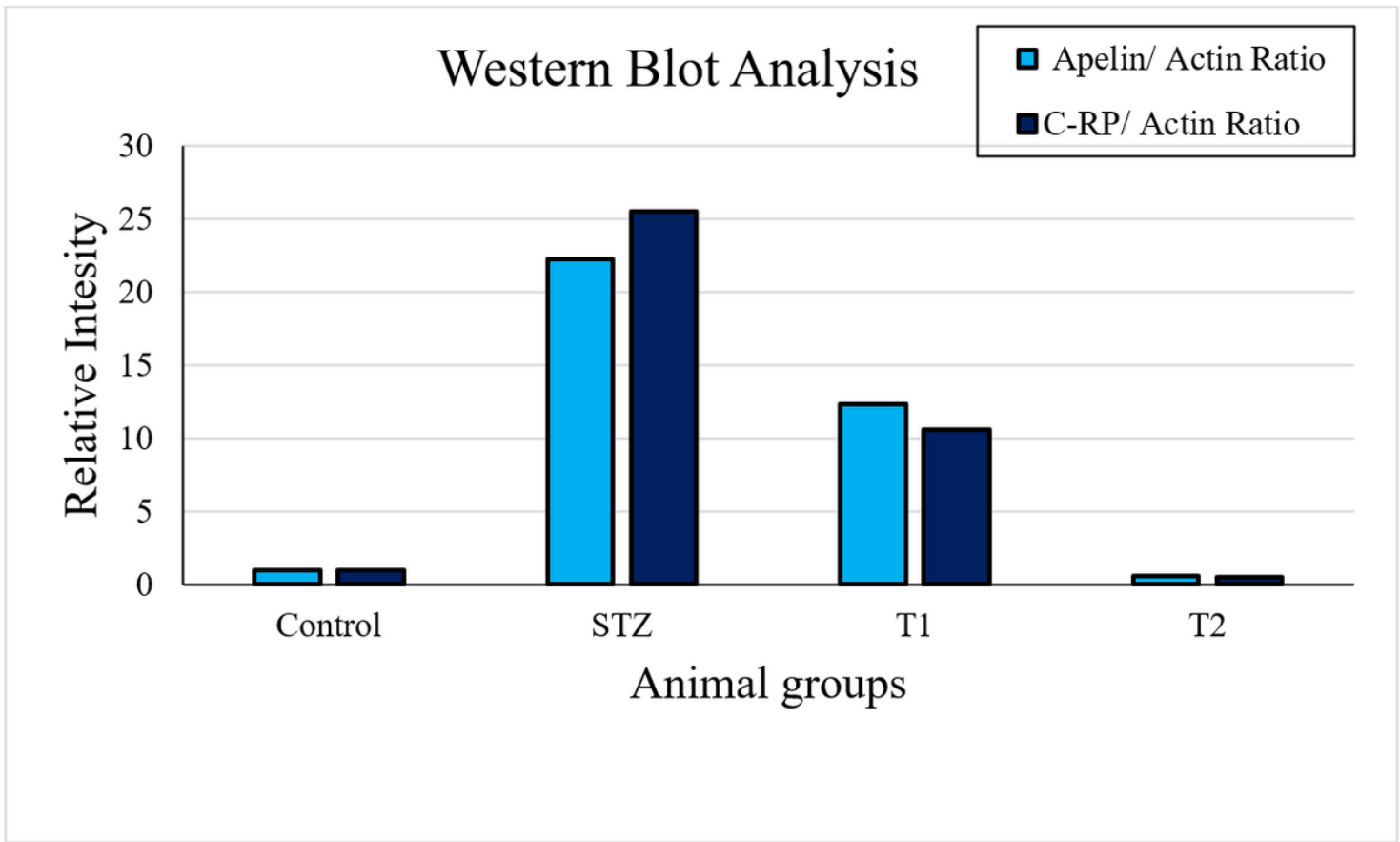


Figure 7

presents the relative intensity of **apelin** and **C-RP** protein expression in plasma samples obtained from the **control**, **STZ**-induced rats, and **T1** and **T2** groups.

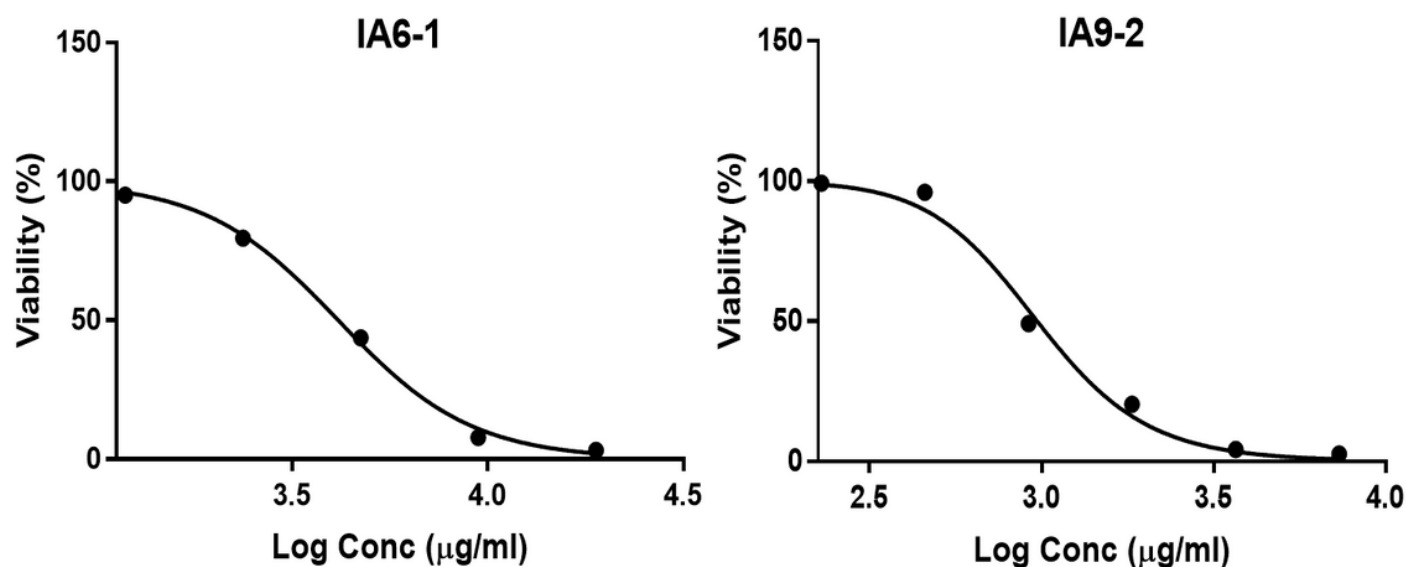


Figure 8

illustrates the relationship between various doses of fraction **IA6-1** (ranging from 1181.25 to 18900 μg/ml) and fraction **IA9-2** (ranging from 228.9 to 7325 μg/ml) and the resulting cell viability of HepG₂ cells, as determined by the **MTT** assay.

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- [WesternBlot.xlsx](#)