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# The DPP-IV Inhibition, Glucose Uptake and Nephroprotective Potential of an Herbal Remedy of *Balbisia Pedunculata* and *Allium Sativum*

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**ABSTRACT:**

This study uses in vitro evaluations to examine the nephroprotective and antidiabetic properties of a herbal formula containing methanol leaf extract of *Balbisia pedunculata* and *Allium sativum* (HF-ASBP). An examination of the herbal formula (HF-ASBP) revealed information on how it affected the activity of DPP-IV and the uptake of glucose by L6 myoblast cells. Although there was a slight drop in blood glucose levels and an increase in glucose absorption in L6 cells, HF-ASBP did have a small antidiabetic impact. However, as there was no significant DPP-IV inhibition, further research is required to determine the precise biochemical mechanisms at play. Concurrently, the nephroprotective capacity of HF-ASBP is examined in relation to cytotoxicity generated by cisplatin in HEK-293 cells. Surprisingly, HF-ASBP showed a strong cytoprotective effect, improving cell survival linked to kidney damage caused by cisplatin. These results establish HF-ASBP as a potentially useful natural herbal formula for nephroprotective treatment by reducing drug-induced kidney damage. As a versatile botanical option for nephroprotective therapies and diabetic management, HF-ASBP exhibits potential. Further research into the particular bioactive chemicals in HF-ASBP, their mechanisms of action, and their potential benefits to reduce the global burden of diabetes and renal disorders is required in light of the complex findings observed in vitro.

**Keywords:** *Allium sativum, Balbisia pedunculata*, Nephroprotective, Diabetes, Antidiabetic

## INTRODUCTION

Diabetes mellitus is a long-term metabolic disease characterised by elevated blood glucose levels. It is a major contributor to numerous issues that affect various organ systems. Diabetic nephropathy is the common term used to describe nephrotoxicity caused by long-term diabetes. One of the many long-term diabetes consequences that dramatically increases morbidity and death is diabetic nephropathy. Kidney failure may result from diabetic kidney disease (DKA), which can gradually deteriorate the renal system. Anomalies in the structure and function of the kidneys are the primary indicators of diabetic nephropathy, and they eventually result in decreased renal function [1-4]. In patients with diabetes, oxidative stress, inflammation, and chronic hyperglycaemia all contribute to the start and progression of nephrotoxicity. Renal

tubule deficiencies could arise from the intricate interaction of these variables. Chronic kidney disease can result from these deficits because they can lead to glomerular hyperfiltration and increased permeability of the glomerular filtration barrier. The three mainstays of modern treatment for diabetic nephropathy are blood pressure management, glycaemic control, and the use of renin-angiotensin-aldosterone system inhibitors. It is clear from the research now available on the treatment of diabetic nephropathy that more modern, effective, and intense therapies are required. Using natural resources is a pragmatic way to develop treatments for the complex pathophysiology of diabetic nephropathy. Numerous phytochemical substances, such as flavonoids, terpenoids, glycosides, phenolic compounds, and polyphenols, are extracted and derived from medicinal plants. These phytochemical substances have demonstrated the ability to lessen and neutralise oxidative stress, inflammation, and fibrosis—the three main causes of nephrotoxicity [5-7]. An increasing amount of study has examined the possible nephroprotective advantages of many plant-based substances, such as curcumin (Curcuma longa), lycopene (found in tomatoes, watermelon, and wine), resveratrol (found in red wine and grapes), and green tea catechins. These phytochemicals have the potential to slow down or even reverse the development of diabetic nephropathy by influencing different signalling pathways that are connected to fibrosis and inflammation. Furthermore, these substances' antioxidant capacity can reduce or neutralise oxidative stress. Literature indicates that nephropathy, diabetes, and cardiovascular disease are significantly correlated. Several of these phytochemical substances have the potential to reduce nephrotoxicity while also protecting the cardiovascular system [3, 5-10]. Promising results from investigations on *Allium sativum* and *Balbisia pedunculata* in labs and with animals have been reported recently in scholarly literature. However, it is crucial to conduct human clinical studies in order to put these discoveries into reality as clinical treatments for individuals.

*Allium sativum* L., often known as garlic, belongs to the Alliaceae family and is the second most commonly used Allium, after onions. As said, it is grown extensively over the world and utilised as a spice, additive, and medicinal herb [11]. The medicinal qualities of garlic are attributed to its increased concentration of sulphur compounds, specifically diallyl disulfide, diallyl trisulfide, S-allylcysteine, and allicin. With differing chemical compositions and quantities of bioactive component, it can be taken either raw (fresh leaves or dried cloves) or processed (garlic oil, garlic extracts, and garlic powder). It has long been recognised as a healthy spice and a well-liked remedy for a range of illnesses and physiological issues [11, 12]. In modern civilization, garlic is used as a therapeutic cure in many different ways. Because of this, researchers from a wide range of disciplines are currently focusing their efforts on figuring out how beneficial garlic is for human health. Researchers looking into the medical qualities of garlic are particularly interested in its broad-spectrum therapeutic effects with low toxicity [12]. Garlic extract possesses antiviral, fungicidal, and antibacterial properties. The chemical components of garlic have been praised by a number of authors for their potential to treat conditions like hyperlipidemia, blood pressure, cancer, atherosclerosis, and cardiovascular disease [12-14].

In many African, South Asian, and Southeast Asian nations, *Balbisia pedunculata*. (Asteraceae) has long been used as a traditional drink to treat liver disorders, diarrhoea,

bronchial catarrh, and dysentery. This is despite reports that the plant may invade numerous crops [15]. Several bioactive chemicals have been effectively extracted from this plant, including puerarin, centaurein, and centaureidin; 8,3′-dihydroxy-3,7,4′-trimethoxy-6-O-β-d-glucopyranosyl flavone; and 6,8,3′-trihydroxy-3,7,4′-trimethoxyflavone [16]. Certain lipid components of this plant have been found, such as lupeol, fucosterol, 30-methyl-28-oxodotriacont-29-en-l-oic acid, methyl 14-oxooctadecanoate, methyl 14-oxononacosanoate, and β-amyrone [16]. Additionally, it has been established that this plant contains phenolic acids, such as guiacol, benzeneacetic, vanilic, and benzoic acids [17]. There is a broad range of biological activity in *Balbisia pedunculata*. The ethyl acetate extract of this plant showed strong allelopathic and larvicidal activities. In terms of pharmacological properties, ethyl acetate extract shown anti-inflammatory, anti-cyclooxygenase, and antioxidant properties, while methanol and ethanol extracts demonstrated anti-hyperglycemic, anti-fungal, anti-leshmanial, and hepatoprotective properties [17]. This herb's acetone extract exhibited anticoagulant, antihepertic, and antibacterial properties [18].

Given the potential therapeutic benefits of *Allium sativum* and *Balbisia pedunculata*, the goal of this study was to bridge the knowledge gap between conventional medicine and modern scientific research [19-21]. This will facilitate the creation of ground-breaking treatments for diabetes mellitus and its associated consequences, such as diabetic nephropathy. Therefore, the purpose of this work is to create a herbal formula using methanol leaf extracts from *Allium sativum* and *Balbisia pedunculata*, and then assess the herbal formula's in vitro nephroprotective and antidiabetic effects using a variety of in vitro mechanistic models.

## MATERIAL AND METHODS

### Chemicals, Drugs, and Biochemical Kits

The providers and vendors of all the drugs, chemicals, reagents, and biochemical kits were vetted and trusted. The chemicals and reagents were analytical grade procured from reputed sources only.

### Collection of plants and Authentication

The candidate medicinal plants, *Allium sativum* and *Balbisia pedunculata* were collected from Kullu district of Himachal Pradesh. Dr. A K gupta, an herbalist with the Department of Botany, identified and authenticated the plant. Herbariums (accession number MK272023/HPK/027 and MK272023/HPK/028) of the plants have been made and deposited in the pharmacognosy laboratory of the university department for future reference.

### Plant Extraction

The plants were dried in the shade for several days and then grinded into coarse powder using a mechanical grinder. The powdered crude drug of each plant (2 kg) was extracted progressively using methanol in cold maceration apparatus for 7 days. All of the obtained plant extracts were concentrated in a rotatory vacuum evaporator at a temperature of < 45°C [22]. Both the extracts - were kept at 2-4°C until use. The two extracts were combined in a 1:1 ration to get the herbal formula (HF-ASBP) which then subjected to total phenolic contents assay.

The percentage yields of all the extracts were calculated using the following standard formula [23]**:**

% Yield= Amount of extract (g)/ Amount of initial dry powder drug (g) ×100

**Preliminary Phytochemical study**

The individual extract and the herbal formula (HF-ASBP) was subjected to preliminary phytochemical screening for detection of phytochemicals present in the extract which includes alkaloids, glycosides, flavanoids, phytosterols, phenols, saponins, proteins, and carbohydrates etc. Standard phytochemical tests were used for the purpose [24].

**Estimating the Total Phenolic Content**

The Folin-Ciocalteu (FC) method was used to assay the total phenolic contents in the herbal formula (HF-ASBP) [25]. Gallic acid as a standard phenolic compound was used and a series of dilutions (110 to 1100 microg/mL) were made for the gallic acid for drawing a standard curve. In the method, sodium carbonate was added to each solution after adding the Folin-Ciocalteu reagent. This mixture was then incubated for 130 minutes and the optical density or absorbance has been measured for each solution at wavelength of 766 nm in a UV-Vis spectrophotometer. The standard curve was used for the total phenolic content estimation in the extracts and expressed as gallic acid (GAE) equivalents per gram of the sample.

**Antidiabetic activity**

***Estimation of Glucose Utilization on L6 Myoblasts***

The techniques outlined elsewhere were utiized to ascertain the usage of glucose in L6 myoblast cells [26]. At a cell density of 4,000 cells/well, L6 cells were added to 104-well growth plates. Until they achieved 95% confluence, these cells were permitted to attach. Two rows were kept cell-free to act as blanks for the glucose utilisation test. The cells were cultivated for five more days after reaching 95 percent confluence in the growth medium, which was changed to DMEM containing 2 percent FBS. The analysis for glucose utilisation was conducted 48 hours before the growth media was refilled. Ten millilitres (15 µg/ml, 25 µg/ml, and 65 µg/ml) of the herbal formula (HF-ASBP) were applied to individual wells for the glucose utilisation experiment. Furthermore, as a positive control, insulin was added to a different column at a concentration of 6 µg/ml. An extra 48 hours were spent incubating the cells in the extract's presence. Following the incubation period, a 26 µl incubation buffer was added to replace the used medium. This buffer was made up of 10 millilitres of glucose, 0.2 percent BSA, and diluted RPMI medium with PBS. After that, the mixture was incubated at 37°C for three more hours. After removing 6 µl of the incubation medium from each well, added the 220 µl of reagent glucose oxidase (Bayer) to each plate well in order to quantify the glucose concentration in the medium. Following a 20-minute incubation period at 37°C, the absorbance at 522 nm was determined using a Multiscan microtitre plate reader (R Systems). The difference between the wells with and without cells was used to compute the glucose usage. The percentage of absorption of glucose was calculated by contrasting it with the untreated control group. The MTT test was used to determine the representative well's cell viability [27].

***Measuring the DPP-IV Inhibition***

The DPP-IV inhibition assay was performed in accordance with the Al-masri et al., 2009 technique [28] with slight modification. In brief, 16 µl of a solution containing human recombinant DPP-IV enzyme (55 µU/µl in Tris buffer) was introduced into the designated wells of a 104-well plate. This was accompanied by the addition of 36 µl of the herbal formula (HF-ASBP) (ranging from 60 µg/ml to 120 µg/ml) or a disease pr activity control (65 µg/ml of positive control, diprotin A). To recruit and start the reaction, 55 µl of Gly-Pro-ρNA (ρNA substrate) at 22 mM dissolved in Tris-buffer had been introduced after a 7-minute incubation at 37°C, followed by an additional 35-minute incubation at the same temperature. Subsequent to the incubation period, the reaction was halted by the addition of 26 µl of a 24% acetic acid solution, and the absorbance at 411 nm was recorded. Furthermore, a sample blank and a blank had been created via replacing 36 µl of the buffer system with HF-ASBPand 16 µl of the buffer with the enzyme, respectively. The inhibition percentage of the enzyme was computed employing the subsequent formula:

% inhibition = (Control Absorbance –Test Absorbance) / Control Absorbance × 100

**Appraisal of Nephroprotective activity**

***Effect of HF-ASBP in cisplatin-induced toxicity in HEK-293 cells***

The human kidney embryonic cell line (ATCC; HEK-293) was utilised in the investigation. The cells had been cultured in DMEM added with a 12% foetal bovine serum inactivated by heat in a carbon dioxide incubation chamber ($CO_2$, 6%) at 37°C. Trypsinization was performed on the cells that were 80–90% confluent, and enough medium was supplied to stop the trypsin's action. Then the cells have been subjected to centrifugation for five minutes at 1400 rpm, the supernatant was discarded, and the pellet was reconstituted in medium before being counted using the Trypan blue exclusion method on a hemacytometer. To obtain the required number of cells, the cells were diluted with medium. In a 104-well microtiter plate with a flat bottom, the final seeding density for cell growth studies was maintained at 9000 cells per well. After seeding for 26 hours, the cells had been kept as treatment added, non-treated, or cotreated for 26 hours with 20µM of cisplatin (CP) and HF-ASBP (10, 20, 40, 80, 160, and 240 µg/mL). Cell viability assay were carried out 26 hours after the start of therapy [29, 30].

***Cell viability test***

Tests for cell viability included the MTT [3-(4, 5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide] assay. This MTT assay offers a delicate evaluation of the regular metabolic condition of cells. Following treatment, the corresponding treated cell wells were filled with MTT solution (6 mg/ml), which was then incubated for three hours. The formazan (dark blue coloured) formed in the end had been dissolved with DMSO solvent followed by measurement of the absorbance at 572 nm using a microtiter plate reader.

**Statistical analysis**

GraphPad Instat version 6 was used to undertake the statistical evaluation of the data. The data were presented as mean ± SD (standard deviation), and the One-Way Analysis of Variance (ANOVA) and Dunnet's multiple comparison test as *Post Test* were used to compare the

various means. When $P < 0.05$, values were deemed to differ substantially (Software, 2013). For plotting the graphs GraphPad Prism Version 7 was used.

## RESULTS AND DISCUSSION

### Phytochemical Screening

To determine the existence of different bioactive chemicals, phytochemical assays were performed on individual plant extracts and HF-ASBP (Table 1). The HF-ASBP tested positive for alkaloids, indicating that these extracts may contain alkaloids. HF-ASBP included carbohydrates, more especially reducing sugars, suggesting a compositional similarity. The HF-ASBP extracts included phytosterols, which are substances that may have health advantages; but did not contain any phytosterols. The HF-ASBP included flavonoids, which are recognised for their antioxidant qualities; also contain terpenoids and proteins. These phytochemical results provide information about the plant's possible medicinal qualities, and differences in the presence of certain compounds suggest that using a variety of solvents is necessary for a thorough phytochemical examination.

**Tablet 1.** The results of the preliminary pharmaceutical screening for all the extracts

| **Phytochemical test** | ***Allium sativum* Methanol Extract (AS-ME)** | ***Balbisia pedunculata* Methanol Extract (BP-ME)** | **Herbal formula (HF-ASBP)** |
|---|---|---|---|
| Alkaloids | + | + | + |
| Carbohydrates | + | + | + |
| Phytosterols | + | + | + |
| Glycosides | + | + | + |
| Flavonoids | + | + | + |
| Saponins | - | + | + |
| Phenols | + | + | + |
| Tannins | + | - | + |
| Proteins | + | + | + |
| Terpenoids | + | + | + |

Herbal formula (HF-ASBP) = (AS-ME + BP-ME)

### Total Phenolic Compounds assay

Gallic acid equivalents (GAE) per gramme of extract were used to express the total phenolic content (TPC) of the different extracts. The regression equations for **HF-ASBP** was $y = 0.0041x + 0.0976$. The formulas provided enabled the determination of the total phenolic content in **HF-ASBP**. With 327.84 GAE per gramme, HF-ASBP had a significant total phenolic content.

### Antidiabetic activity

#### *Glucose Utilization in L6 Myoblast*

At all concentrations examined, HF-ASBP exhibited only minimal efficacy in reducing blood glucose levels in L6 myoblast cells (refer to Figure 1). In comparison to the majority of cases in the treated control group, HF-ASBP (113.90±2.94 percent) also exhibited a minor enhancement in absorption of glucose by L6 cells, although this effect was notably weak at the

maximum tried concentration (65 µg/ml), where it reached 100 percent. In contrast, a positive control, insulin (6 µg/ml) had been employed and demonstrated a more substantial encouragement of glucose uptake in L6 cells, yielding a retort of 141.62±3.42 percent. As depicted in Figure 1, both the extract and insulin treatments on L6 cells, as evaluated by the tetrazolium based MTT technique of assay, showed no indication of possible damage. Additionally, it was noted that, in contrast to the control, the extract and insulin significantly affected L6 cell proliferation for glucose absorption at every concentration examined.

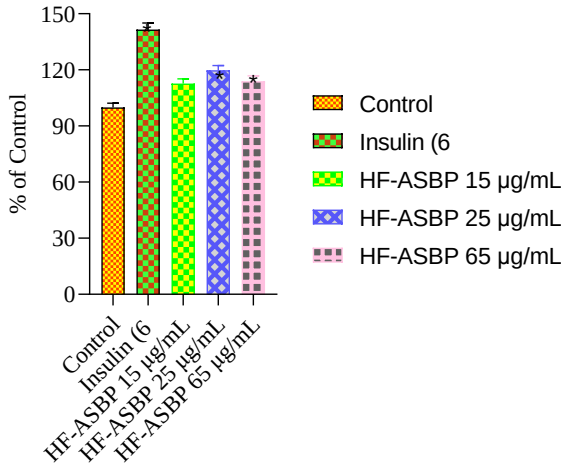


**Figure 1.** Effect of HF-ASBP on the absorption of glucose by L6 myoblasts. For 48 hours, the cells were treated with the herbal blend at either a fixed concentration or varying concentrations.

***Inhibition Assay of DPP-IV***

When HF-ASBP was evaluated at each concentration, it showed a discernible but somewhat less inhibition than diprotin A, the positive control, which showed an inhibition of 86.78 ±1.84 percent against DPP-IV activity (as illustrated in Figure 2). However, there was a notable and significant suppression observed with the extract compared to the non-treated control.

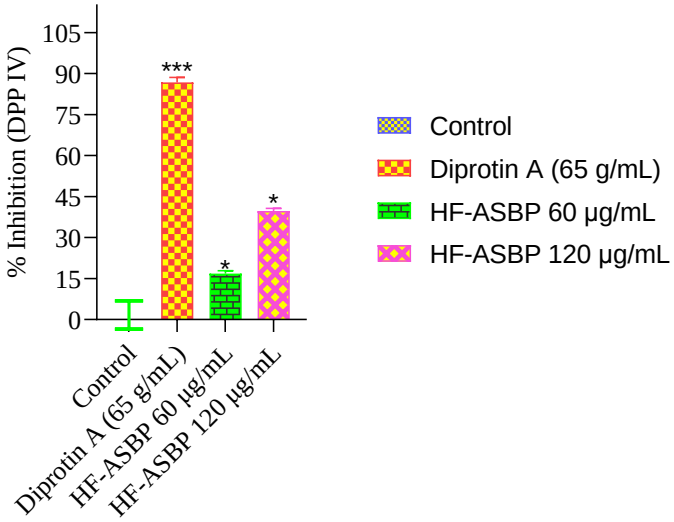


**Figure 2.** The impact of HF-ASBP on % DPP-IV activity inhibition.

**Nephroprotective activity**

***Result of impact of HF-ASBP in nephrotoxicity in HEK-293 cell Line induced by cisplatin***

The effectiveness of the HF-ASBP in terms of nephroprotection was appraised using nephrotoxicity model in human embryonic kidney (HEK-293) cells induced by cisplatin. The viability of cell assay was performed to determine the nephron-cytoprotective effect of HF-ASBP in human embryonic kidney cells treated with cisplatin. The cells were exposed to varying HF-ASBP concentrations (10, 20, 40, 80, 160, and 240 µg/mL) for a whole day, either without or with 25 µM of Cisplatin. The HF-ASBP therapy by itself has no appreciable detrimental effects on cell viability. Treatment with cisplatin dramatically reduced the viability of the cells in comparison to a normal control ($P<0.001$). Cells treated with HF-ASBP and CP together greatly increased in viability. When HF-ASBP therapy was applied instead of cisplatin control, the cell viability increased by 10–33 percent. 10.782 µg/mL was found to be the EC50 (Figure 3).

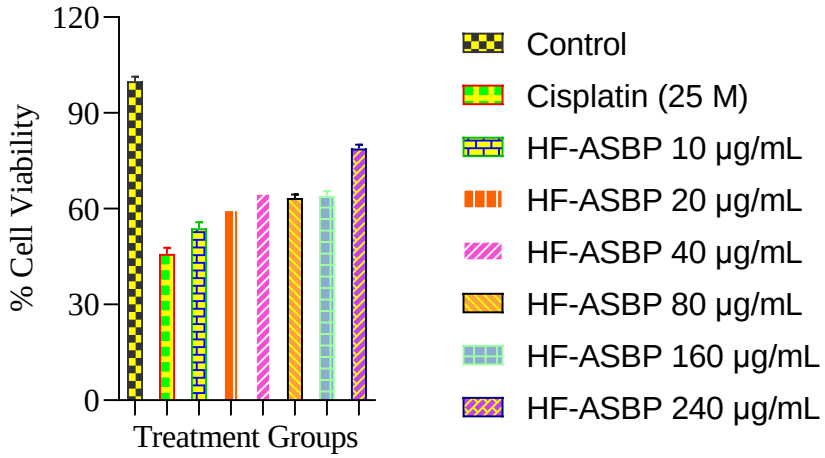


**Figure 3.** Impact of HF-ASBP on cisplatin-induced nephrotoxicity in HEK-293 cells.

**DISCUSSIONS**

Through in vitro tests, the study investigated nephroprotective and antidiabetic properties of HFUB-ME, providing insight into possible therapeutic uses. The HF-ASBP showed marginal potential in reducing blood glucose levels in L6 myoblast cells during the evaluation of its antidiabetic action, with a minor increase in glucose uptake, especially at lower concentrations. Remarkably, the impact was not as strong as the untreated control system at the uppermost dosage tested (65 µg/ml). As a positive control, insulin, on the other hand, showed greater stimulation of glucose uptake, suggesting that HF-ASBP may have a less significant effect than insulin. Crucially, the MTT assay showed no evidence of toxicity by HF-ASBP on L6 cells, indicating the substance's safety profile when it comes to glucose utilisation.

Additionally, the study looked into the ways HF-ASBP inhibited DPP-IV enzyme in the metabolism of glucose. It was unexpected that HF-ASBP did not significantly decrease DPP-IV activity at any of the tested doses. The absence of inhibition compared to diprotin A, the control, raises questions about the mechanisms underlying the antidiabetic effects of HFUB-ME. This study investigated the effect of HF-ASBP in the damage caused by cisplatin in HEK-

293 cells. First, checked if HF-ASBP itself had any harmful effects on the cells and found that it didn't cause any harm, suggesting that renal cells can use it safely and effectively. When cisplatin was used alone in a positive control cell, it significantly decreased cell viability and caused damage. However, when HF-ASBP was used alongside cisplatin, it increased cell viability by up to 33%, showing a protective effect against cisplatin-induced damage. The study observed this effect at a concentration of 10.782 µg/mL. The results for this research work demonstrated important findings regarding the protective effect of HF-ASBP. HF-ASBP demonstrated weak DPP IV activity but significant effects on glucose utilization for diabetes. The herbal formula revealed significant protective action against Cisplatin induced nephrotoxicity. Further studies will be needed to fully explore the huge therapeutic potential of this ferbalk formula (HF-ASBP). For the same future research may include evaluation of nephroprotective and antidiabetic activities in suitable animal models *in vivo* followed by the isolation of the responsible phytochemical components in HF-ASBP.

## CONCLUSIONS

In summary, this research has explored the potential uses of HF-ASBP containing methanol extract of leaf of *Allium sativum* and *Balbisia pedunculata* for kidney protection and managing diabetes. HF-ASBP had a modest effect on lowering blood sugar levels compared to insulin, especially in L6 myoblast cells' glucose utilization. The limited inhibition of DPP-IV activity raises interesting questions about how HF-ASBP works for diabetes. Since natural extracts have a mix of bioactive phytochemicals, more studies are needed to understand how HFUB-ME's components work together. For kidney protection, HF-ASBP showed a positive effect on damaged HEK-293 cells from cisplatin. The increase in cell survival and changes in cell shape suggest HF-ASBP could help reduce kidney injury. This finding opens the door to exploring HF-ASBP as a natural treatment, especially for drug-induced kidney issues. However, it is crucial to note the limitations of lab-based research, even though HF-ASBP shows promise. These discoveries have implications for human health, prompting further research through live organism studies (in vivo) and, eventually, clinical trials. Overall, HF-ASBP seems promising for renal protection and managing glucose levels. Despite the mentioned limitations, this research provides a strong reason to study the specific phytochemicals in HF-ASBP, how they work, and their potential for treatment. Using natural resources like *Allium sativum* and *Balbisia pedunculata* could lead to new ways of managing renal issues and diabetes, addressing the need for safe and effective therapies in these areas.

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