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Synergistic Hepatoprotective Effect of Methanolic Extracts of *Urtica Dioica* LINN. Leaves and Silymarin CCL_4 - Induced Hepatic Damage in $HEPG_2$ Cells

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Abstract

In this current study, the methanolic leaves extract of Urtica dioica Linn. (UDLE-M) was tested for its hepatoprotective and antioxidant properties. The extract's hepatoprotective activity was assessed in HepG2 cell lines against CCl4-causedhepatic injury. Additionally, in order to assess the antioxidant activityof UDLE-M in vitro, the assessment of total antioxidant activity was looked at.The synergistic effect of UDLE-M + Silymarin was also investigated in HepG2 cell lines. The extract exhibited noteworthy antioxidant activity, matching that of common antioxidant chemicals such as BHA (Butylated hydroxyanisole) and α-tocopherol, according to the data. A notable hepatoprotective profile was also noted, with an enhanced level of % cell viability suggesting that the dose of 110 µg/mL would result in the highest hepatoprotection. A significant synergistic hepatoprotective effect had also been observed for UDLE-M + Silymarin.

Keywords: Urtica dioica, antioxidant, Total antioxidant activity, carbon tetrachloride, hepatoprotective.

1. INTRODUCTION

Over the past few years, natural antioxidants have been used widely. The majority of diseases, including those related to the heart, osteoporosis, inflammation, degenerative diseases, cancer, etc., are associated with the generation of reactive nitrogen species, reactive oxygen species (ROS), and oxidative stress concept[1]. Unsaturated fatty acids are the main target of free radicals when it comes to cellular membrane damage. This can result in lipid peroxidationof bio-membranes, receptor activity, a diminution in fluidityof membrane, a decrease in antioxidant defense enzymes, and degradation of cellular membrane protein. Ultimately, the inactivation or death of the cell is caused by these damaging processes[2]. Therefore, the detrimental and pathogenic effects of free radicals can be countered by antioxidants. Generally speaking, antioxidants cleanse the body and scavenge free radicals. In addition to being produced naturally by bodily metabolism, free radicals can also be picked up from the environment. Free radicals are a byproduct of many redox reactions. These can be non-free radical species such as hydrogen peroxide (H_2O_2) and singlet oxygen ($\bullet O_2$), or they can be oxygen radicals such as superoxide radical (O_2-) and hydroxyl radical (•OH) [3]. The antioxidant defense system's enzymes, such as catalase, superoxide dismutase, and glutathione peroxidase, capture and eliminate these free radicals. Deficits in some vitamins, increased lipid peroxidation, a decrease in antioxidant defence enzymes, and an excess of free radical generation are thought to be the causes of oxidative-stress and have been associated with numerous clinical ailments[4]. Plants and other natural resources have been utilised for health maintenance and quality of life enhancement since ancient times. Plants have been used by humans for aeons as food and drink, as well as in cosmetics, colours, and medications. Many plant species have been employed as an essential source of antioxidants, antibiotics, and other beneficial substances, depending on the indigenous-traditional folklore medical utilization in various societies all overthe world. Recent years have seen a rise in the focus of research on plants and chemicals produced from plants that may have bioactivity [5]. Numerous plant species are regarded as abundant providers of antioxidant compounds. Around the world, local groups and traditional healers utilise a variety of plants and their parts for their therapeutic qualities.

The hepatic system, liver is a vital organ-system in the physiological system that is involved in several metabolic functions, digestion, and detoxification. It can, however, be affected by a variety of illnesses that might impair its functionality and cause serious health issues. Liver problems comprise aextensivearray of illnesses, for instancecirrhosis, fatty liver disease, liver cancer, and hepatitis. These conditions are frequently brought on by things like alcoholism, viral infections, obesity, and exposure to toxins. There has been an increase in attention in the potential of traditional medicinal plants as hepatoprotective agents in recent years due to the hunt for efficient treatments for liver ailments. Throughout history, traditional medical systems have employed medicinal plants—also referred to as herbal remedies or phytomedicines for therapeutic purposes.They provide a wealth of bioactive chemicals with a variety of pharmacological characteristics, including the potential for hepatoprotection. Substances known as hepatoprotective agents can aid in preventing liver injury and fostering liver regeneration. In this regard, medicinal plants are quite promising because they provide a natural and frequently less intrusive method of promoting liver health [6-8].

The hepatoprotective qualities of a number of medicinal plants have been investigated, as has their efficacy in treating liver diseases in both clinical and laboratory settings. The milk thistle plant's extract, silymarin, is among the most well-known examples. Silymarin's capacity to shield the liver against a variety of assaults, including alcohol, poisons, and some drugs, has been the subject of much investigation. It is thought to function by lowering inflammationand oxidative stress in the liver by functioning as an antioxidant and anti-inflammatory agent.Another noteworthy hepatoprotective plant is *Phyllanthus amarus*, also referred to as the "stonebreaker" plant. This herb has long been used in traditional medicine to treat ailments of the kidneys and liver. According to studies, *Phyllanthus amarus* may have hepatoprotective benefits by lessening liver damage, preventing the spread of the hepatitis

virus, and promoting the regeneration of liver cells [9, 10]. The plant Curcuma longa yields turmeric, a spice that includes curcumin, a bioactive substance. Due to its anti-inflammatory and antioxidant characteristics, curcumin has drawn interest and is being researched for possible liver disease relief. Based on available research, curcumin appears to be a good choice for hepatoprotection as it may help lower oxidative stress and liver inflammation. *Tinospora cordifolia*, often known as guduchi, is another herbaceous plant with hepatoprotective properties. It is thought to function by lessening liver damage and improving the liver's detoxification activities. Traditional Ayurvedic medicine has employed guduchi to enhance liver function and promote liver health [11-15].

Moreover, it is impossible to exaggerate the significance of holistic approaches in the management of liver diseases. Combining dietary changes, lifestyle adjustments, and the use of hepatoprotective medicinal plants can help to preserve liver health and reduce the risk of liver problems. Antioxidants included in fruits and vegetables, such green tea, artichokes, and carrots, can, for example, enhance the hepatoprotective effects of medicinal herbs. People can take proactive measures to protect their liver function by combining these dietary choices with therapeutic plant-based therapies. In conclusion, liver diseases represent a serious health risk and are becoming more commonplace throughout the world. Because of their hepatoprotective qualities, medicinal herbs provide a safe, natural option for controlling and avoiding liver illnesses. Studies on a variety of medicinal plants, including Guduchi, *Phyllanthus amarus*, turmeric, and silymarin, show promise in defending the liver against harm, lowering inflammation, and promoting liver regeneration. It is becoming more and more clear how important medicinal herbs are for supporting liver function as we work toward holistic approaches to treatment. We will be able to prevent and treat liver problems in the long run by having a better understanding of the mechanisms and effectiveness of these plants through additional study and clinical trials. Using lifestyle changes in conjunction with medicinal plants can be an effective way to improve liver health and general wellbeing [14-18].

An ancient Ayurvedic herb recognized by many as Vrishchhiyaa-shaaka is *Urtica dioica* Linn. (Family: Urticaceae). Additionally, it goes by the names Bichu Butti in PunjabiandHindi, Shisuun in Kumaon folk language, Anjuraa in Unani, and in English asStinging Nettle [19, 20]. The plant, which thrives throughout the Indian subcontinent and numerous South Asian countries, is an perennial as well as annual herb. It has long been recognized as a therapeutic herb worldwide. It can also be found in Himachal Pradesh, Arunachal Pradesh, and Assam [19, 20]. Traditionally, the medicinal plant was utilized as an anthelminthic, emmenagogue, blood purifier, protective, and diuretic[19, 20]. Additionally, it is used to treat menorrhagia, haematuria, jaundice, and nephritis [19]. Furthermore, this medicinal plant has been shown to include linolenic acid, lutein and its isomers, lectins, neoxanthin, beta-carotene and its isomers, lycopene, and violaxanthin, according to earlier research. In addition to its antidiabetic and antihypertensive properties, the herb is also said to have natriuretic and diuretic properties [21-28].
The goal of this work was to examine the antioxidantand hepatoprotectiveactions of *Urtica dioica Linn* leaf methanolic fraction. Along with determining the total phenolic content, *Urtica dioica Linn* leaf methanolic fractionunderwent preliminary phytochemical screening. Determining the extract and silymarin's combined hepatoprotective effects in $HepG_2$ cells was also interesting. Additionally, this study aims to provide a convincing defense for the plant's use in traditional folklore and ayurvedic medicine to treat and manage anassortment of ailments, together with liver diseases.

2. MATERIALS AND METHODS

Plant

Herbalist Dr. S. K. Sharma of Joginder Nagar, Mandi, Himachal Pradesh, verified the authenticity of leaves taken from the Solan area of the Indian state of Himachal Pradesh. For future use, the Pharmacognosy department received a voucher specimen herbarium (BK/DS1/16/2021).

Drugs and chemicals

E. Merck (India) Limited supplied chemicals such carbon tetrachloride (CCl_4), methanol, ammonium thiocyanate, trichloroacetic acid (TCA), gallic acid, α-Tocopherol, butylated hydroxyanisole (BHA), and dimethylsulphoxide (DMSO). We obtained linoleic acids, ascorbic acid, and malondialdehyde (MDA) from Loba Chem Company located in India. All of the reagents and solvents utilized, including petroleum ether and Tween 80, were of analytical grade and readily available in the market (SRL Mumbai, Himedia, E. Merck India). A. S. Pharmaceuticals, Baddi, Himachal Pradesh, arranged for Silymarin to be sent as gift samples.

Preparation of extracts

After being shade-dried, the leaves of *Urtica dioica* Linn. were ground into powder. Methanol was used as the solvent to extract the mechanically grinded and powdered plant leaves using a Soxhlet apparatus, and they were then concentrated in a vacuum. About 5.3 g of dried methanolic extract of *Urtica dioica* Linn. leaves (UDLE-M) were produced from 60 g of dried leaf material (Yield, 8.83 percent).

Preliminary and initial phytochemical evaluation

Using chemical techniques in accordance with the methodology suggested elsewhere [29], preliminary phytochemical screening was done on the extract in powder form as well as on the methanolic extract to identify the phytochemical ingredients.

Measuring and calculatingthe total phenolic content

The total soluble phenolic content of the plant extract (UDLE-M) was determined using the Folin–Ciocalteau technique, with gallic acid acting as a reference phenolic component [30]. 50 milliliters of distilled water were added to around 2.0 milliliters (20 milligrams) of extract in a volumetric flask. After thoroughly mixing the mixture above, two milliliters of the Folin-Ciocalteau reagent were added. The liquid was allowed to stand for four hours with sporadic shaking after adding 4.0 ml of 3 percent sodium carbonate after three minutes. The mixture's absorbance at 761 nm was measured in a spectrophotometer (UV -1801 Shimadzu, Japan). The total phenolic compound content was expressed in milligrams per gram of extract. Equation generated from the equation of the regression line of the standard gallic acid graph was used to express the concentration of total phenolic components in the extract as grams of gallic acid equivalent (GAE):

$Y = 0.0024x + 0.055,$
$r^2 = 0.9736$
Where, y = absorbanceandx = concentration.

***In vitro* antioxidant activity**

Evaluating the Total antioxidant activity

The thiocyanate approach, which was previously reported with some modifications, was used to measure the extract's total antioxidant activity [31]. Ten milligrams of extract were so dissolved in twelve milliliters of water. To 3.5 ml of linoleic acid emulsion-containing phosphate buffer of potassium (pH 7.2, 0.05 M), various concentrations of UDLE-M (60-260 µg/mL) or standard samples were added (0.05 M, pH 7.2). 18.6 g Tween-40, 16.6 µl linoleic acid, and 0.05M potassium phosphate buffer make up a five-milliliter linoleic acid emulsion (pH 7.2). Likewise, 3.2 milliliters of potassium salt phosphate buffer and 3.2 milliliters of linoleic acid emulsion are present in the 5.5 milliliter control (0.06 M, pH 7.3). At 37°C, the mixture was allowed to incubate in the dark. Following five minutes of vigorous stirring, the mixture was subjected to a reaction with thiocyanate compound and $FeCl_2$at consistentinterludesincubation throughout. The absorbance at 502 nm was then measured in a spectrophotometer (UV -1801 Shimadzu, Japan) to estimate the peroxide value. Peroxides were produced during the oxidation of linoleic acid. These substances convert Fe2+ into Fe3+. The subsequent Fe3+ ions combine to form a combination with

SCN−, whose absorbance peaked at 502 nm. Consequently, elevated absorbance signifies elevated oxidation of linoleic acid. As blank samples, the solutions devoid of extract or standards were employed. The average of three duplicate analyses is used to determine all total antioxidant activity results. The following formula was used to determine the percentage of lipid peroxidation inhibition:

Percentage Inhibition = $(X_0 – X_t/ X_0) \times 100$

where Xt represented the absorbance while the sample was present and X0 represented the control absorbance. Every test was run 3 times, & a plot displaying the mean ± SD data was created. α-Tocopherol was the usual antioxidant that was employed.

Evaluationof hepatoprotective activity *In vitro*

To assess the hepatoprotective potential of UDLE-M, the hepatic cell line $HepG_2$ was used in an *in vitro* model of CCl_4-induced hepatotoxicity. In order to better understand UDLE-M's potential as a hepatoprotective agents, this study sought to ascertain how well it protects liver cells ($HepG_2$)from CCl_4-induced damage.

Cell line

The human liver cell line $HepG_2$ has been the subject of intensive research and analysis, greatly advancing our understanding of liver bio-physiology, metabolism of xenobiotic and drugs, and many liver-related ailments. Since these cells were first extracted from a 15-year-old Caucasian man's liver tumour in 1970, they have recognized to be a treasuredsource for *in vitro* research [32]. $HepG_2$ cells are highly significant for many reasons. The main functioning liver cells, known as fully developed hepatic cells, can be demarked by the characters they display. These hepatic cells are capable of performing many different tasks, including the synthesis of proteins, the digestion of fats, and the metabolism of medications. Using $HepG_2$ cells, researchers study liver-specific processes viz., drug induced toxicity, replication of hepatitis virus, and the impact of numerousconstituents on hepaticwell-being. Furthermore, since $HepG_2$ cells are so simple to cultivate and maintain, they can be used in a wide range of laboratories and research centers. They offer a dependable and replicable model for examining liver-related processes and can be employed to assess the toxicity or effects of possible drug candidates on functionality of the liver. All things considered, the HepG2 cell line is a vivacious means for biological & bio-medical and physiological research work, especially in the expanses of toxicity, drug development,hepato-physiology, and hepatology.Its ease of use and liver-specific features make it an extremely valuable means for acquiring knowledge about liver bio-physiology and improving our understanding of liver diseases and drug interactions [32, 33].

Assessment of Hepatoprotective activity of UDLE-M usinghepatic($HepG_2$) Cell Line

Hepatoprotective effectiveness was evaluated by screening for protection against CCl_4-induced damage in human liver-derived $HepG_2$ cells[34] and determined by utilizing the tetrazolium assay to evaluate mitochondrial production [35]. DMEM complemented with 10% serum of newborn calf was widely utilised to cultivate and subculture $HepG_2$ cells as monolayers. The cells employed in this study were those that had undergone a batch culture lasting 12 days beforehand. After that, the cells were taken out and plated at 28,000 cells per well density of on 104-well Nunclon microtiter plates. They were then permitted to sit for 25-26 hours in a humidified atmosphere at 37°C with 5-6 % carbon monoxide. The extract, the medium by itself, or both were then added to the cells at different quantities, along with CCl_4 as the toxicant (medium containing 1-2 percent CCl_4) (as normal)[34]. It has been shown that silymarin dosages exceeding 260 µg/ml and UDLE-M dosages exceeding 110 µg/ml are toxic to cells; dosages within the range of 30 to 110 µg/ml were used. At the end of the trial, By evaluating the HepG2 cells' viability, the cytotoxicity was evaluated using the MTT reduction assay [35]. After an hour of incubation, the test solution from each well was withdrawn, and 55 µl of MTT generated in MEM without phenol red was added in its stead. The plates were quickly shaken and then incubated at 37°C for four hours in a

humid atmosphere with 6% CO_2. Once the supernatant is discarded, add 60 µl of propanol, the formazan that had been produced was dissolved by gently shaking the plates. A microplate reader was used to calculate the absorbance at 540 nm.

Statistical analysis

A linear regression approach had beenemployed to graphically estimate the total contentof flavonoid. The mean ± SD for three animal replicates was used to express the data for the antioxidant and hepatoprotective activities. The average of three separate analyses was used to represent all other data. For comparison analysis student 't' test was employed to statistically analyze the data and a p value of 0.05 or less has been set as significant.

3. RESULTS

Preliminary and initial phytochemical evaluation

Following a preliminary and initial phytochemical evaluation with several chemical techniques, the powdered leaves and the methanolic extract (UDLE-M) were found to include phytosterols, proteins, amino acids, saponins, flavanoids, tannins, hydrolysable tannins, and phenolic compounds. The results of the alkaloid test were negative.

Determination of total phenolic content (TPC)

Phenols and phenolics are significant phytoconstituents that have the ability to scavenge free radicals because of their hydroxyl groups. However, high concentrations of phenolics and related chemicals are not always associated with antioxidant benefits [30]. The TPC of the *Urtica dioica* Linn. leaf methanolic extract (UDLE-M) was also tested. The present analysis determined that there were 208.11 µg of total phenolics per milliliter of GAE/g extract (Figure 1).

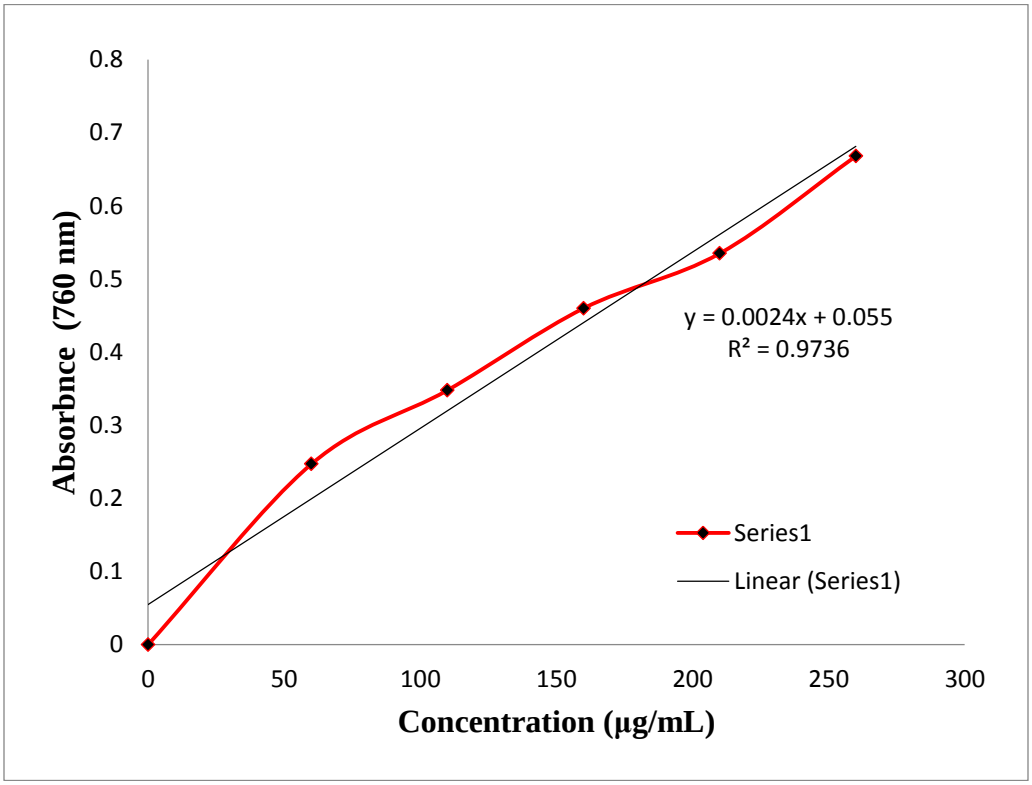

Figure 1. Estimated amount of TPC in the UDLE-M.

***In vitro* antioxidant action**

Total antioxidant actionestimation in a systemof linoleic acid

The noted thiocyanate method was utilized to measure the plant extract's (UDLE-M) overall antioxidant activity. At 260 µg/ml, the extract exhibited strong and efficient antioxidant activity. UDLE-antioxidant M's activity first rose as the incubation period increased, but when the incubation period increased again, the activity of the compound decreased. Figure 2 illustrates the effect of the extract's 260 µg/ml concentration on the linoleic acid emulsion-peroxidation.Compared to α-Tocopherol at 260 µg/ml, the extract exhibited greater antioxidant activity at that dose. At the 12-hour mark, the extract's percentage inhibition of peroxidation in the linoleic acid system was 54.85±1.46%. Additionally, at the 12-hour time point, the percentage inhibition of the 260 µg/ml concentration of α-Tocopherol was reported to be 21.59±1.76%.

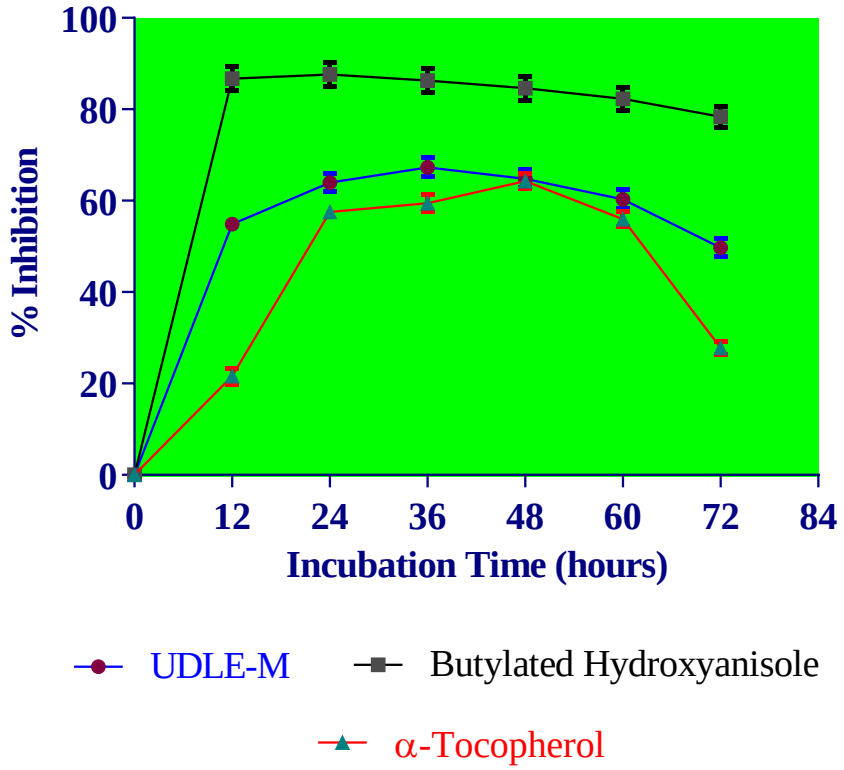

Figure 2. The results of total antioxidant activity of UDLE-M.

***In vitro* hepatoprotective activity of the UDLE-M**

Appraising the hepatoprotective action in HepG$_2$ Cells

After being exposed to CCl4, the vitality of the HepG$_2$ cells was measured and found to be 17.68 ± 2.37 percent. In comparison to the group that was inebriated with CCl_4, these outcomes met statistical significance ($P < 0.001$). Interestingly, when exposed to different concentrations of UDLE-M, concentration-dependent increases in the viability of the inebriated cells were observed. Table 1 demonstrates that at UDLE-M dosages (30 to 110 µg/mL), the percent cell viability varied from 74.75 ± 3.54 to 93.94 ± 3.76 percent.Furthermore, the percentage viability rose dramatically oncetreated the HepG2 cells with UDLE-M at 90 and 110 µg per millilitre as opposed to the conventional treatment with Silymarin as standard at 260 µgper millilitre ($P<0.01$), enhancing the cell viability demonstrating the higher efficacy of UDLE-M.

Table 1. When $HepG_2$ cells are exposed to CCl_4, the UDLE-M displays hepatoprotective properties.

Treatments	**Concentration (µg/ml)**	**% Cell Viability**
Normal Control	-	100
Positive Control (CCl_4 intoxicated)	-	$17.68 \pm 2.37^{*}$
CCl_4 (1%) intoxicated + Treated with Standard Silymarin	260	$93.91 \pm 3.94^{\#}$
CCl_4 (1%) + UDLE-M treated	110	$93.94 \pm 3.76^{\#}$
	90	$91.12 \pm 3.96^{\#}$
	70	$89.85 \pm 3.68^{\#}$
	50	$78.67 \pm 2.19^{\#}$
	30	$74.75 \pm 3.54^{\#}$
An average of three determinations and 3 replicates (n = 3); * denotes significant compared to normal cells at $p < 0.001$; # = denotes significant compared to the CCl4-toxicated cells at $p < 0.01$.		

Synergistic Effects of UDLE-M and Silymarin

After being exposed to CCl4, the vitality of the HepG2 cells was measured and found to be 17.68 ± 2.37 percent. In comparison to the group that was inebriated with CCl4, these findings were significant statistically ($P < 0.001$). Interestingly, when exposed to different concentrations of UDLE-M + Silymarin, the viability of the intoxicated cells increased in a concentration-dependent manner. Table 2 and Figure 3 show that at concentrations of UDLE-M + Silymarin from 30 to 110 µgper millilitre, the cell percentage viability oscillated starting 82.88 ± 3.43 to 98.99 ± 3.65 percent.Furthermore, the percentage vitality increased significantly ($P<0.01$) oncethe HepG2 cells had been treated with UDLE-M + Silymarin at 90 and 110 µgper millilitre as opposed to the conventional therapy with Standard hepatoprotective Silymarin at 260 µgper millilitre. This indicates that UDLE-M + Silymarin is substantially more effective at promoting cell viability. The combination of UDLE-M with Silymarin therapy resulted in a considerable increase in cell viability, suggesting a stronger synergistic impact.

Table 2. UDLE-M and Silymarin exerts synergistic hepatoprotective effects on CCl_4 - toxicated $HepG_2$ cells.

Treatments	**Concentration (µg/ml)**	**% Cell Viability**
Normal Control	-	100
Positive Control (CCl_4intoxicated)	-	$17.68 \pm 2.37^{*}$
CCl_4 (1%) intoxicated + Treated with Standard Silymarin	260	$93.91 \pm 3.94^{\#}$
CCl4 (1%) intoxicated + Treated with [UDLE-M + Silymarin]	110	$98.99 \pm 3.65^{\#}$
	90	$94.09 \pm 3.87^{\#}$
	70	$91.91 \pm 3.57^{\#}$
	50	$87.79 \pm 2.23^{\#}$
	30	$82.88 \pm 3.43^{\#}$
An average of three determinations and 3 replicates (n = 3); * denotes significant compared to normal cells at $p < 0.001$; # = denotes significant compared to the CCl_4-toxicated cells at $p < 0.01$.		

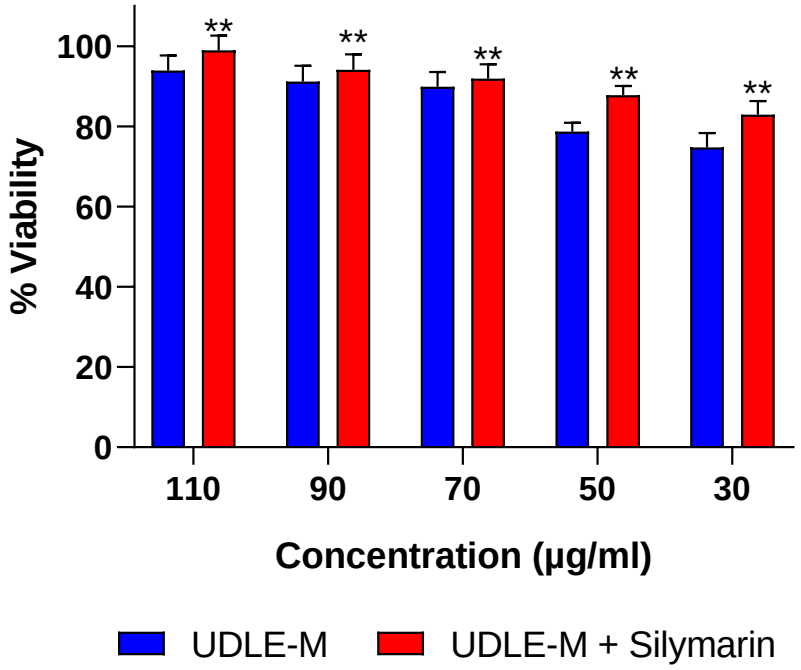

Figure 3. Comparative depiction of synergistichepatoprotective effects of UDLE-M and Silymarin.

4. DISCUSSION

Carbon tetrachloride (CCl_4) is a well-known hazardous material that has been widely used in scientific settings to cause liver damage in experimental mice. Although CCl_4 is no longer frequently seen in daily life due to its extreme toxicity, it is nevertheless a vital tool for examining the causes and pathophysiology of liver injury as well as prospective therapies. CCl_4-induced liver injury has been the subject of extensive research because it closely resembles a number of features of liver problems in humans. Following injection, the liver's cytochrome P-450 enzymes convert CCl_4, resulting in the production of highly reactive free radicals called methyl trichloro radicals ($\bullet CCl_3$). These free radicals cause a series of harmful processes in the liver cells, such as lipid peroxidation, which results in oxidative stress.Hepatocytes undergo cellular necrosis, mitochondrial malfunction, and membrane degradation as a result of this process. Moreover, chronic exposure to CCl4 can cause liver tissue to gradually scar, which makes it a suitable model for researching liver cirrhosis and fibrosis. The attraction of immune cells to the site of damage is accompanied by inflammation, a characteristic symptom of liver injury. Researchers employ the liver injury model caused by CCl_4 to assess the effectiveness of hepatoprotective medicines, antioxidants, and antifibrotic drugs as well as to investigate possible therapeutic treatments. The fundamental molecular mechanisms behind liver damage and repair are made more understandable by this model. It is important to understand that, even with the utility of the CCl_4 model, there can be limitations on how successfully it can be applied to real liver diseases. Because CCl_4 is extremely hazardous to humans and because different liver illnesses may entail diverse pathways of liver injury, it cannot be used in clinical research. Because of this, it is important to workout caution when extrapolating model results to human circumstances. It is likely a well-researched experimental paradigm that offers valuable insights into the mechanisms underlying oxidative stress, fibrosis, inflammation, and liver injury caused by CCl_4. It is important to recognise that although useful in the creation of new treatments for liver illnesses, it is not a perfect copy of the complexity of human liver pathologies, which calls for additional research in therapeutically relevant settings.

Within the framework of this investigation, it was found that CCl_4 seriously damaged $HepG_2$ cell lines [36-38], and UDLE-M, on the other hand, showed notable dose-dependent hepatoprotective benefits. As demonstrated by studies for total antioxidant activity, UDLE-M also demonstrated noteworthy antioxidant activity. Typically produced from one-electron reductions of molecular oxygen molecules, reactive oxygen species are linked to oxidative stress and can produce in lipid peroxidation, DNA damage, and GSH oxidation,—protein oxidationprocesses in which iron may play a major role [39-41].In-depth research on these markers was done for this study. Oxidative stress is a significant pathophysiological component that underlies a wide spectrum of disease conditions, as emphasised by numerous research [42-44]. The antioxidant and liver protective qualities of UDLE-M in hepaticinjury in $HepG_2$ cell lines were successfully proven in this work, with antioxidant potential serving as a critical predictor of the most likely hepatoprotective pathway. In the linoleic system, the study discovered that UDLE-M demonstrated strong antioxidant action.

Additionally, the hepatoprotective activity of UDLE-M + Silymarin was assessed in $HepG_2$ cells and contrasted with the outcomes of UDLE-M alone. This allowed for the assessment of UDLE-M and Silymarin's synergistic hepatoprotective action, with the findings pointing to this combination's benefits. This discovery broadens the scope of these chemicals' potential to shield the liver from harm. In conclusion, the CCl4 model demonstrated common features of liver injury, such as leukocyte infiltration, severe necrosis, fibrosis, and moderate to hepatic hydropic degeneration.However, at the investigated dosages, UDLE-M significantly protected the liver, consistent withanearlier study employing hepatic $HepG_2$ cells. Silymarin and UDLE-M together showed a synergistic hepatoprotective effect. These results highlight UDLE-significant M's hepatoprotective and antioxidant qualities as well as its potential for use in conjunction with Silymarin to provide a viable strategy for liver protection.

5. CONCLUSIONS

To sum up, the experimental model of liver injury caused by carbon tetrachloride has been extremely helpful in illuminating the intricate processes behind fibrosis, inflammation, oxidative stress, and liver damage. Due to its severe toxicity in humans and the variety of mechanisms behind different liver problems, carbon tetrachloride (CCl4) is a dangerous material with limited application to clinical liver diseases; yet, it has remained an essential tool for researchers. This model allows for a thorough investigation of possible liver disease treatments by closely simulating key aspects of human liver disorders. The research described here also clarifies UDLE-hepatoprotective M's characteristics, in particular, its strong antioxidant activity and dose-dependent effects on liver protection. Moreover, the examination of the combination of UDLE-M and Silymarin shown a positive synergistic hepatoprotective effect. These results open up a potentially new direction for liver protection and the study of medicinal plants' potential to treat liver diseases. The CCl_4 model has limitations that should be carefully considered before directly applying it to clinical practise. However, this study highlights the significance of continuing research in clinically relevant settings to close the knowledge gap between human liver pathologies and experimental models. To sum up, the utilization of the CCl_4-induced liver damage model in conjunction with the assessment of medicinal plants such as UDLE-M and Silymarin presents a multifaceted strategy for augmenting our comprehension of liver health and promoting prospective therapies for liver ailments.

Declaration of Interest

The authors declare no conflict of interest.

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