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In vitro Mechanistic Study on Antiradical and Hepatoprotective Activity of *Ruta graveolens* leaf extract

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ABSTRACT:

This study aimed to explore the health benefits of RGLE, a herbal extract made from the leaves of *Ruta graveolens*, processed using methanol extraction. We conducted several in vitro tests to determine RGLE's potential in promoting health, particularly focusing on its ability to fight oxidative stress and support liver health. One of the well-known methods we used was the DPPH radical scavenging assay. The results were quite promising, showing that RGLE has a strong capability to neutralize harmful free radicals, which are known contributors to oxidative stress. Furthermore, we examined how RGLE could protect liver cells using a model where HepG2 cells were damaged with carbon tetrachloride, a common chemical used to study liver injury in the lab. The findings were encouraging—RGLE significantly protected these liver cells from damage caused by the chemical or toxicant, CCL4. Overall, our study indicated the functionality of RGLE as powerful antioxidant and a protector of liver cells. This suggests that RGLE could be a valuable natural option for managing oxidative stress and improving liver health. Further research, especially in clinical settings, could provide deeper insights into how RGLE can be used effectively for health enhancement and disease prevention.

Keywords: Antiradical, DPPH radical, Hepatoprotective, *Ruta graveolens*, Herbal extract

1. Introduction

Hepatic injury is frequently linked to disruption of the liver's essential role in the body's regulation of metabolism, secretion, storage, and detoxification processes. Reactive oxygen species (ROS) can be dealt with by liver cells by a variety of compensatory methods, one of which is the production of antioxidant proteins such glutathione peroxidase (GSHPx), catalase, and superoxide dismutase (SOD). Cu–Zn, Mn–SOD, catalase, GSHPx, and GSH reductase (GR) are examples of enzymatic antioxidant systems that work by directly or sequentially removing ROS to stop their activities. Oxidative injury is the result of an imbalance between the oxidative forces and antioxidant defense mechanisms. This imbalance has been linked to a number of diseases, including liver cirrhosis, diabetes, cancer, and atherosclerosis [1].

One of the biggest and most important organs in the human body, the liver is critical for controlling several physiological functions that are necessary for existence. This covers the

production of the proteins required for blood coagulation, metabolism, immune system operation, nutrition storage, and detoxification. Its optimal operation is essential to preserving general health and wellbeing. These activities are significantly compromised by liver impairment, which can be brought on by a variety of circumstances including chronic viral hepatitis, excessive alcohol intake, certain drugs, and exposure to toxins. Damage to the liver makes it more difficult for it to properly digest nutrients, filter blood, and eliminate toxins from the body. This can cause an accumulation of waste materials and poisons that can impair brain function; this is referred to as hepatic encephalopathy [2, 3].

Furthermore, liver damage can result in cirrhosis, or scarring, which impairs liver function and raises the risk of serious side effects such liver failure and portal hypertension. It is impossible to exaggerate the significance of liver health. It plays a key role in the metabolism of fats and carbohydrates, which is essential for controlling energy levels. Drugs and other compounds are also metabolised by the liver, which is essential for detoxification and the effectiveness of medications. Moreover, albumin, the most prevalent protein in blood plasma, is synthesised by the liver and is crucial for maintaining osmotic equilibrium, which controls blood pressure and volume. Lifestyle decisions including consuming alcohol in moderation, keeping a healthy weight, having safe sexual relations, and using medications appropriately all contribute to preventing liver disease. Liver health can also be enhanced by regular exercise and a diet high in fruits, vegetables, proteins, and healthy fats. Additional protective measures include vaccinations against hepatitis A and B. Given its extensive influence on health, it follows that the liver's optimal function is critical. It is crucial to safeguard the liver from harm in order to avoid major health problems, highlighting the importance of knowledge and preventative actions in order to preserve liver health [1, 4].

Under healthy conditions, ROS is constantly produced and efficiently removed by a variety of intracellular and extracellular antioxidant mechanisms. Damage to cellular macromolecules (DNA, lipids, and proteins) as well as other tiny antioxidant molecules is frequently caused by the unchecked generation of ROS. Superoxide anion radical O2–, hydrogen peroxide (H2O2), alkoxyl (RO), peroxyl (ROO), hydroxyl radical (OH), and hypochlorous acid (HOCl) are the most significant ROS. Reactive nitrogen species (RNS) that are not oxygen species, such as peroxynitrite and nitric oxide (NO), are likewise highly bioactive. A crucial route in many diverse biological systems that are unconnected to one another is the free radical reaction. Among many ways of chemical-induced injury, the critical class of reaction is production of free radical intermediates which trigger a network of multifarious disturbances [5-7].

Lipid peroxidation and other oxidative insults are the primary mechanisms by which the majority of hepatotoxic substances harm liver cells. Because of its special metabolism and crucial function in removing chemicals from the portal circulation, the liver is vulnerable to oxidative stress, xenobiotic toxicity, and medication toxicity. The two different metabolic pathways in the liver are carried out by GSH-peroxidase and cytochrome p-450. Currently, medications that affect the p-450 enzyme mechanism are used to treat hepatotoxicity. These medications can either induce or inhibit the metabolic activity of enzymes, such as ciprofloxacin, amiodarone, carbamazepine, and phenobarbital, or phenytoin [4, 6, 7].

The hepatoprotective properties of naturally occurring extracts or compounds, as well as their methods of action, have garnered a lot of attention lately. Here, we investigated the hepatoprotective potential of a herbal extract using carbon tetrachloride cytotoxicity as a test against HepG2 cells. HepG2 cell toxicity generated by chemicals is a good in vitro model for hepatotoxicological medication evaluation by examining several cytotoxic endpoints. Because HepG2 cells retain several specialised tasks typical of normal human hepatocytes, such as the synthesis and secretion of plasma proteins, they have been used to study the metabolism and toxicity of medications [8-11].

Many traditional treatments are suggested for the treatment of liver problems in the lack of dependable contemporary hepatoprotective medications. Numerous plants have been shown to have hepatoprotective properties, including Andrographis paniculata, Tridax procumbens, and Silybum marianum. Numerous bioactive substances, including as phenols, flavonoids, steroids, and terpenoids, are found in plants. These phytoconstituents have many pharmacological qualities, including anti-inflammatory, antiviral, anti-proliferative, and anti-carcinogenic effects, in addition to their nutritional importance. Phenolic, flavonoid, and polyphenolic chemicals obtained from plants are thought to help prevent disorders linked to oxidative stress [12, 13]. The perennial herb rue, or Ruta graveolens, is native to the Balkan Peninsula but is now grown as a garden plant all over the world. Rue, which has a strong, acrid flavour and perfume, has been utilised historically in both culinary and medical applications. The plant has yellow summer blossoms and bluish-green, fern-like leaves. It can reach a height of approximately 0.6 metres. Traditional medicine has employed Ruta graveolens for a number of ailments. It is claimed to have anti-inflammatory, antifungal, and antispasmodic properties. It was once used to treat conditions like eye problems, as a poisoning remedy, and to soothe digestive issues. Additionally, rue is employed in many ethnic rites and superstitions as a means of warding off evil. However, due to its strong active chemicals, which can be poisonous, Ruta graveolens should be used with caution. The plant includes a number of phytochemicals, such as alkaloids and flavonoids, which, if consumed in large amounts, can result in severe photosensitivity reactions, gastrointestinal problems, and neurological symptoms. Due to its possible toxicity and intense flavour, its usage in homoeopathic medicines and as a decorative plant has become more prevalent than in traditional culinary applications. Considering all these facts, this present study aimed to evaluate the antiradical and hepatoprotective activity of a methanol leaf extract of *Ruta graveolens* in various mechanistic in vitro experimental models.

2. Material and Methods

Drugs, Chemicals and Reagents

M Sea Pharmaceuticals, located in Paonta Sahib, Himachal Pradesh, India, generously provided Silymarin and all standard drugs as complimentary samples. Other chemicals, drugs, and reagents were acquired from Lab India in Delhi, India, and Sigma Chemicals Company in St. Louis, Missouri. All additional chemicals and reagents used were of analytical-grade and obtained from reputable suppliers.

Preparation of Extracts

Ruta graveolens was arranged from Herbal Traders, Kullu, Himachal Pradesh, India. Its validity was confirmed by Prof. S. K. Sharma, a botanist from Himachal Pradesh, India. The herbarium of the same institute received a sample of the plant. The plant's fresh, air-dried leaves were ground into a powder using an electric grinder. The solvent was filtered after the powder was periodically shaken during a 7-day soaking in methanol. After gathering the filtrate, it was extracted using the Soxhlet device. After hoover drying, the extract was stored at -4 ^{o}C for subsequent use (yield: 15.8%). Eventually, the codename for the herbal extract was RGLE.

Preliminary phytochemical screening

Chemical methods were used to identify the phytochemical components of the crude drug's powder and methanolic extract in accordance with previously proposed methodology [14].

Determination of total phenolic content

With the Folin-Ciocalteau reagent, total soluble phenolics in the plant's leaf extract were calculated using the standard phenolic compound gallic acid [15]. A 46 ml of distilled water were used to dilute approximately 1.0 ml of extract solution, which contained 10 mg of extract in a volumetric flask. The Folin-Ciocalteau reagent (about 1.0 ml) was added and well mixed. After adding 3.0 ml of 2% sodium carbonate after three minutes, the mixture was left to stand

for three hours while being periodically shaken. The mixture's absorbance was determined using a spectrophotometer (UV -1601 Shimadzu, Japan) set at 760 nm. The mg/g of extract was used to express the total phenol content. Based on an equation derived from the standard gallic acid graph, the content of total phenolic components in the extract was expressed as grammes of gallic acid equivalent (GAE):

$Y = 0.0033x + 0.108$, $R^2 = 0.9447$

Where, Y was the absorbance and x was the concentration.

In vitro assessment of antiradical activity

DPPH (1-1-diphenyl- 2-picryl hydrazyl) radical scavenging activity

DPPH• used the aforementioned methods to assess the extract's ability to scavenge free radicals [16]. Based on the plant extract's ability to scavenge the stable DPPH free radical activity, its antioxidant activity was investigated. Forty microliters of extract solution with varying doses (0.02 - 2 mg/ml) were mixed with 300 microliters of an ethanolic solution of DPPH (0.05 mM). Fresh DPPH solution was made and stored at 4°C in the dark. After adding 2.7 ml of 96% ethanol, the liquid was forcefully shaken. After allowing the mixture to stand for five minutes, the absorbance at 517 nm was determined using spectrophotometry. The absorbance was adjusted to zero using ethanol.. Additionally, a blank sample with the same concentration of DPPH and ethanol was made. Every determination was made three times. The following formula was used to determine the evaluated samples' radical scavenging abilities, represented as a percentage of inhibition. (AB-AA) / AB] x 100 = Percent (%) inhibition of DPPH activity where AA and AB represent the test and blank sample's respective absorbance values. The concentration of sample needed for 50% inhibition was calculated and expressed as the IC50 value for each of the test solutions, and a percentage inhibition versus concentration curve was drawn.

***In vitro* appraisal of hepatoprotective activity**

In the traditional model of CCl4-induced hepatotoxicity, the hepatic (HepG2) cell line was employed to evaluate the in vitro hepatoprotective effectiveness.

Cell line

HepG2 cells are an immortalized cell line derived from human liver carcinoma. These cells are widely used in scientific research, particularly in studies related to liver biology, metabolism, and the effects of drugs and toxins on liver function. HepG2 cells originate from a liver tumor in a 15-year-old Caucasian male, and they exhibit hepatocyte-like characteristics, making them a valuable model for hepatocellular function and pathology [17]. One of the key features of HepG2 cells is their ability to produce albumin, a major plasma protein synthesized by the liver. This characteristic is particularly important as it indicates that these cells retain some of the functional properties of normal hepatocytes, despite their cancerous origin. Additionally, HepG2 cells express a variety of liver-specific enzymes and transporters, which are crucial for studying liver metabolism, including the metabolism of drugs and other xenobiotics.

In toxicology, HepG2 cells are employed to study the hepatotoxicity of various substances. Researchers use these cells to assess the toxicity of chemicals, pharmaceuticals, and herbal supplements, examining how these substances affect cellular viability, DNA, and protein synthesis. HepG2 cells are also used to investigate mechanisms of liver diseases such as steatosis and hepatitis, providing insights into disease progression and potential therapeutic targets. Moreover, HepG2 cells are instrumental in the study of viral hepatitis, particularly hepatitis B and C, as they are susceptible to infection by these viruses. This makes them a useful tool for exploring viral life cycles, host-virus interactions, and the efficacy of antiviral drugs. In cancer research, HepG2 cells are used to study the molecular and cellular mechanisms of hepatocarcinogenesis. Researchers utilize these cells to investigate the roles of various genes and signaling pathways in liver cancer development and progression. Additionally, they serve

as a model for testing anticancer drugs and studying resistance mechanisms. Overall, HepG2 cells are a versatile tool in biomedical research, offering insights into liver function, disease, and therapeutics. Their widespread use underscores their importance in advancing our understanding of liver-related health issues [17, 18].

Hepatoprotective activity in HepG$_2$ Cell Line

Hepatoprotective efficacy was evaluated by screening for protection against CCl4-induced damage in human liver-derived HepG2 cells [19] and determined by using the tetrazolium assay to evaluate mitochondrial production [20]. [19]. [20]. Using well-maintained HepG2 cells, the extracts' hepatoprotective potential was assessed using the technique outlined in our earlier paper. Silymarin was employed as a conventional hepatoprotective medication and CCl4 as a hepatotoxicant. In a 96-well tissue culture plate, confluent HepG2 cells were cultivated at a density of 5 × 104 cells/well using growth media (EMEM + 10% FBS) and incubated for a whole night. Following incubation, cells were exposed to various extract concentrations for two hours. Afterward, CCl4 (1 mM) was added, and the cells were incubated for a further two hours. Following incubation, the treated cells were rinsed with DPBS and placed in growth media containing MTT. After removing the medium, DMSO was used to dissolve the formazan crystals. At 570 nm, the optical density was observed.

Statistical Data Analysis

Data are expressed as mean ± standard deviation (SD) of two independent experiments. Each experiment was performed in triplicates. Statistical differences between the treatments and the control were evaluated by one-way analysis of variance (ANOVA) and Dunnett's multiple comparison tests using GraphPad Prism 5.0 (GraphPad Software, Inc., San Diego, CA). The criterion for statistical significance was *P < 0.05 and **P < 0.01..

3. Results

Preliminary phytochemical screening

Proteins and amino acids were found in the powdered leaves that underwent initial phytochemical screening using several chemical techniques. These included phytosterols, saponins, flavanoids, tannins, hydrolysable tannins, and phenolic compounds. The alkaloid test yielded a negative result. Comparable phytochemical screening of the plant's powdered leaves in methanolic extract revealed the presence of proteins, amino acids, alkaloids, saponins, phytosterols, flavanoids, and tannins. However, contrary with the powdered leaves, hydrolyzable tannins were not found.

Table 1. Results of preliminary phytochemical screening

Category tested	**Findings**
Carbohydrates	+
Alkaloids	+
Steroids and sterols	+
Glycosides	+
Saponins	+
Flavanoids	+
Tannins and phenolic compounds	+

Triterpenoids	–
Proteins and amino acids	+

'+' = presence and '-' = absence

Determination of total phenolic content

The majority of plant sources' antioxidant properties come from chemicals of the phenolic class. Because of their hydroxyl groups, phenols are essential plant components that have the capacity to scavenge. However, the benefits of antioxidants are not always correlated with high concentrations of phenolics [15] The extract's total phenolic content was assessed. The study's total phenolic content was determined to be 9.97 mg GAE/g of extract. There was no association found in this study between antioxidant activity and phenolic content.

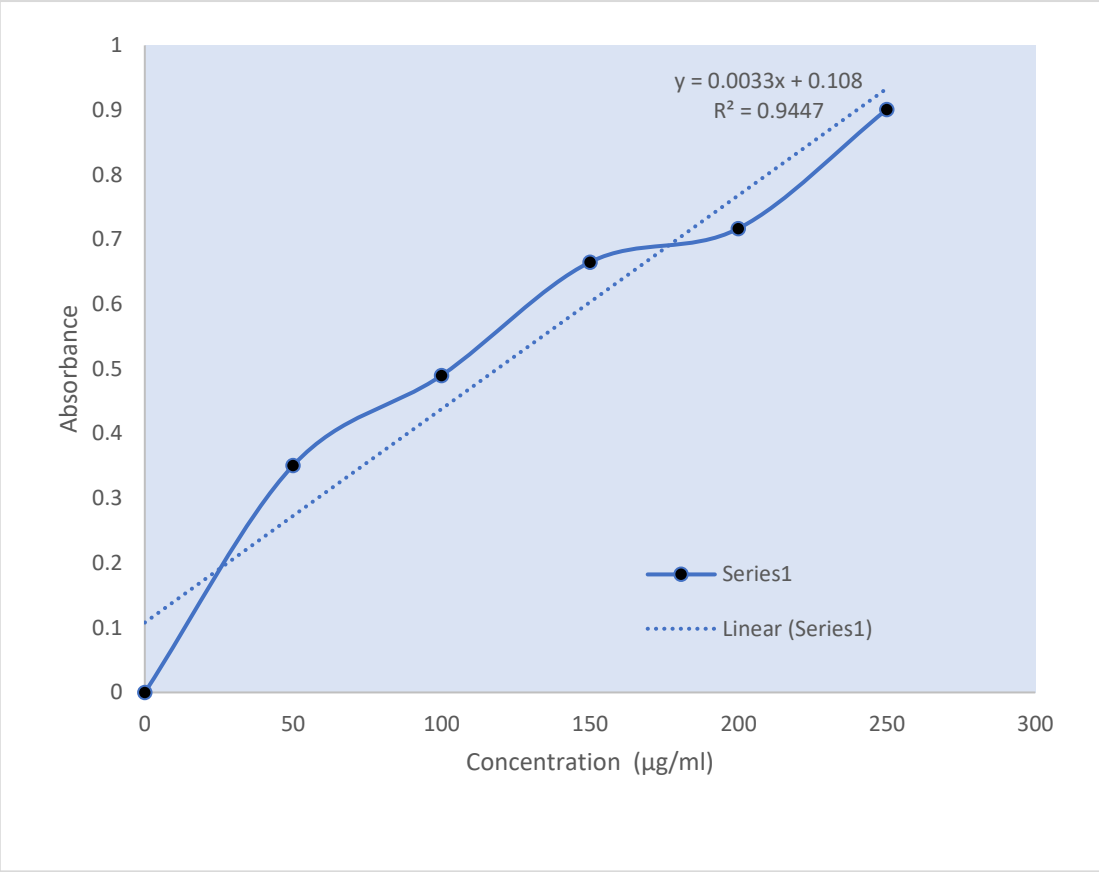

Figure 1. Total phenolic content in the extract

***In vitro* evaluation of antiradical activity**

Appraising the DPPH (1-1-diphenyl- 2-picryl hydrazyl) radical scavenging activity

In this study, the antioxidant capabilities of butylated hydroxytoluene (BHT) and RGLE were assessed using the DPPH radical scavenging assay. The data presented includes the percentage of DPPH radical scavenging activity at different concentrations (10, 50, 100, 150, 200, and 250 µg/mL) for both BHT and RGLE, alongside the standard deviation values for each concentration. These results provide insightful comparisons between the efficacies of a synthetic antioxidant (BHT) and a natural extract (RGLE) in neutralizing free radicals. At the lowest concentration (10 µg/mL), BHT exhibited a scavenging activity of 11.56%, which is slightly lower than that of RGLE at 14.56%. This initial data suggests that RGLE might be more effective than BHT at lower concentrations. However, as the concentration increases to

50 µg/mL, BHT and RGLE show scavenging activities of 45.52% and 50.75%, respectively, indicating a continuing trend where RGLE performs marginally better than BHT. At 100 µg/mL, a notable increase in activity is observed for both antioxidants, with BHT displaying 74.01% scavenging activity and RGLE showing slightly less effectiveness at 66.77%. This switch suggests that BHT becomes more effective than RGLE at higher concentrations. As the concentration increases further to 150 µg/mL, 200 µg/mL, and 250 µg/mL, BHT consistently shows higher scavenging activity compared to RGLE. Specifically, at 200 µg/mL and 250 µg/mL, BHT nearly reaches a plateau with scavenging activities of 95.78% and 96.03%, respectively, compared to 83.16% and 91.86% for RGLE. The superior performance of BHT at higher concentrations could be attributed to its stable phenolic structure, which is highly effective in donating hydrogen atoms to neutralize free radicals. BHT is a well-established synthetic antioxidant used widely in the food and cosmetics industries due to its stability and effectiveness. In contrast, the fluctuating performance of RGLE at different concentrations might reflect the complex nature of natural extracts, which contain a variety of phytochemicals contributing to their overall antioxidant capacity. These compounds may have synergistic or antagonistic interactions that affect their radical scavenging abilities. The practical applications of these findings are significant for industries looking to incorporate antioxidants into their products. While BHT offers robust and consistent performance, its synthetic origin may not be preferable for products marketed as natural or organic. On the other hand, RGLE, with its natural origin and effective scavenging ability at lower concentrations, might be more suitable for such products, despite its slightly lower efficacy at higher concentrations.

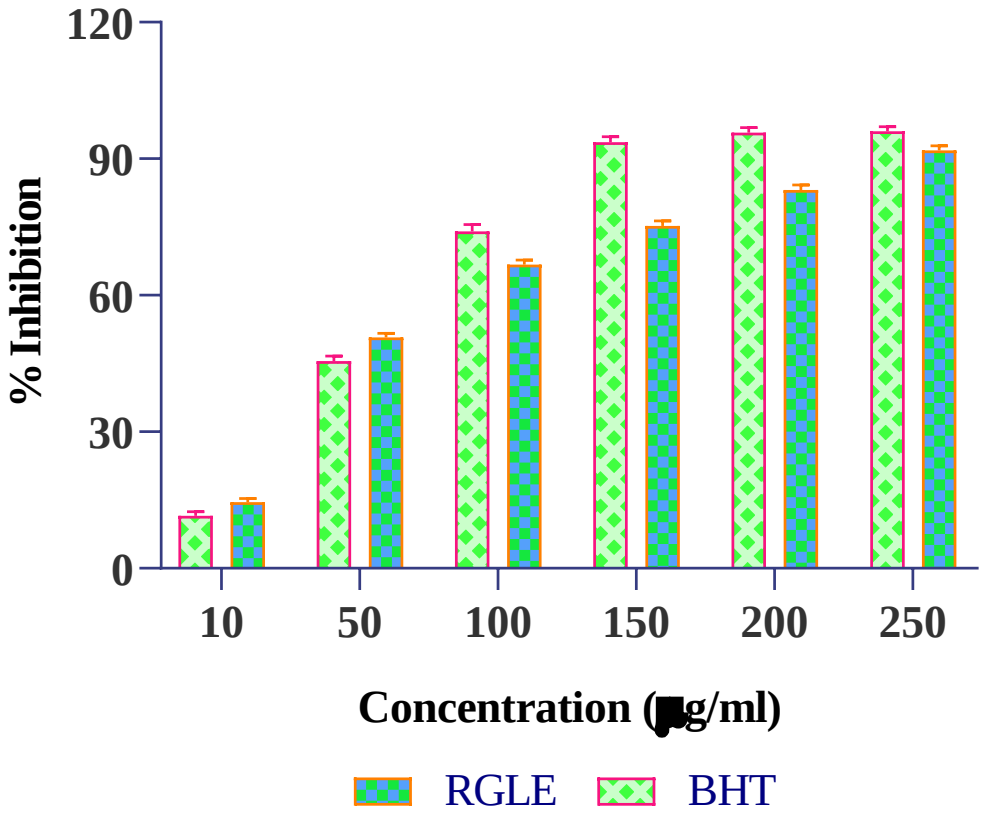

Figure 2. Depicts the ability to DPPH scavenging RGLE

The reference substance (BHT) and the RGLE were found to have estimated IC50 values of 70.27 and 78.63 µg/ml, respectively [21]. In inference, both BHT and RGLE demonstrate effective DPPH radical scavenging activities, with BHT showing greater efficacy at higher concentrations and RGLE performing better at lower concentrations. The choice between using BHT or RGLE should consider factors such as the desired product positioning (synthetic vs. natural) and the specific antioxidant needs based on product formulation. Further research into the mechanisms of action and interactions of the phytochemicals within RGLE could enhance its application potential in natural product formulations.

***In vitro* hepatoprotective activity**

Appraising the hepatoprotective action in HepG$_2$ Cells

The presented study investigates the hepatoprotective efficacy of RGLE in HepG2 cells subjected to carbon tetrachloride (CCl4)-induced toxicity. This model is significant as CCl4 is a well-documented hepatotoxin commonly used in experimental research to simulate chemical-induced liver damage. The data encapsulates cell viability percentages across various treatment groups, highlighting the potential protective effects of RGLE compared to the standard treatment of silymarin, a known hepatoprotective compound. The control group, which was not exposed to CCl4, maintained 100% viability, serving as the benchmark for assessing cell health. Exposure to CCl4 drastically reduced cell viability to 21.48%, indicating significant hepatocellular damage. This set the stage for evaluating the protective effects of the treatments. The CCl4-treated cells that were subsequently treated with silymarin (260 µg/ml) showed a viability of 94.09%, demonstrating silymarin's effectiveness in mitigating CCl4-induced toxicity. This finding is consistent with the well-documented liver-protective properties of silymarin, which acts primarily through antioxidant mechanisms and enhancing cellular defence systems.

Interestingly, RGLE treatment at a concentration of 105 µg/ml resulted in a slightly higher cell viability (96.75%) compared to silymarin, suggesting that RGLE might offer comparable, if not superior, hepatoprotective benefits. The hepatoprotective effect of RGLE was also evident at lower concentrations, with viability decreasing in a dose-dependent manner as the concentration of RGLE was reduced from 105 µg/ml to 25 µg/ml. The findings suggest that RGLE possesses potent hepatoprotective properties which could be attributed to its rich composition of phenolic compounds and antioxidants. These compounds potentially mitigate oxidative stress and enhance cellular antioxidant capacity, thereby protecting HepG2 cells from CCl4-induced cytotoxicity. The dose-dependent increase in cell viability with increasing RGLE concentrations supports the hypothesis that higher doses of RGLE are more effective in combating liver toxicity.

Table 1. The RGLE has hepatoprotective effects on $HepG_2$ cells that have been CCl_4-toxicated.

Treatment Groups	**Concentration in µg/ml**	**% Viability**
Control System	-	100
CCl_4 treated	-	21.48 ± 2.07^{a}
CCl4 (1%) + Standard Silymarin treated	260	94.09 ± 3.01^{b}
CCl4 (1%) + RGLE treated	105	96.75 ± 3.13^{b}
	85	94.47 ± 3.11^{b}
	65	89.88 ± 3.33^{b}
	45	85.91 ± 2.42^{b}
	25	82.53 ± 3.43^{b}

Five determinations on average, 4 replicates (n = 5); a = $p < 0.001$, in contrast to normal cells; b = $p < 0.01$, contrasted with the CCl_4-toxicated cells.

The superior performance of RGLE at 105 µg/ml, in comparison to the standard silymarin treatment, is particularly noteworthy. This could implicate specific components within RGLE that have distinct mechanisms of action or synergistic effects that are more effective than silymarin alone. Further biochemical analyses would be required to identify these active compounds and elucidate their mechanisms. These results underscore the potential of RGLE as a natural therapeutic agent for liver protection, which could be particularly appealing given the growing consumer preference for natural over synthetic health solutions. However, while RGLE shows promise in vitro, clinical studies are necessary to confirm its efficacy and safety in humans. Further research should also explore the specific bioactive components responsible for RGLE's hepatoprotective effects. Understanding these mechanisms in greater detail could lead to optimized formulations and dosing strategies, enhancing the therapeutic potential of RGLE. Additionally, comparative studies involving other known hepatoprotective agents could help position RGLE within the broader context of liver health interventions. In inference, this study highlights the hepatoprotective potential of RGLE in a model of CCl4-induced liver damage in HepG2 cells. RGLE not only demonstrated comparable efficacy to the standard silymarin treatment but, at certain concentrations, exceeded it. These findings suggest a promising role for RGLE as a natural alternative for liver protection, meriting further investigation in clinical settings.

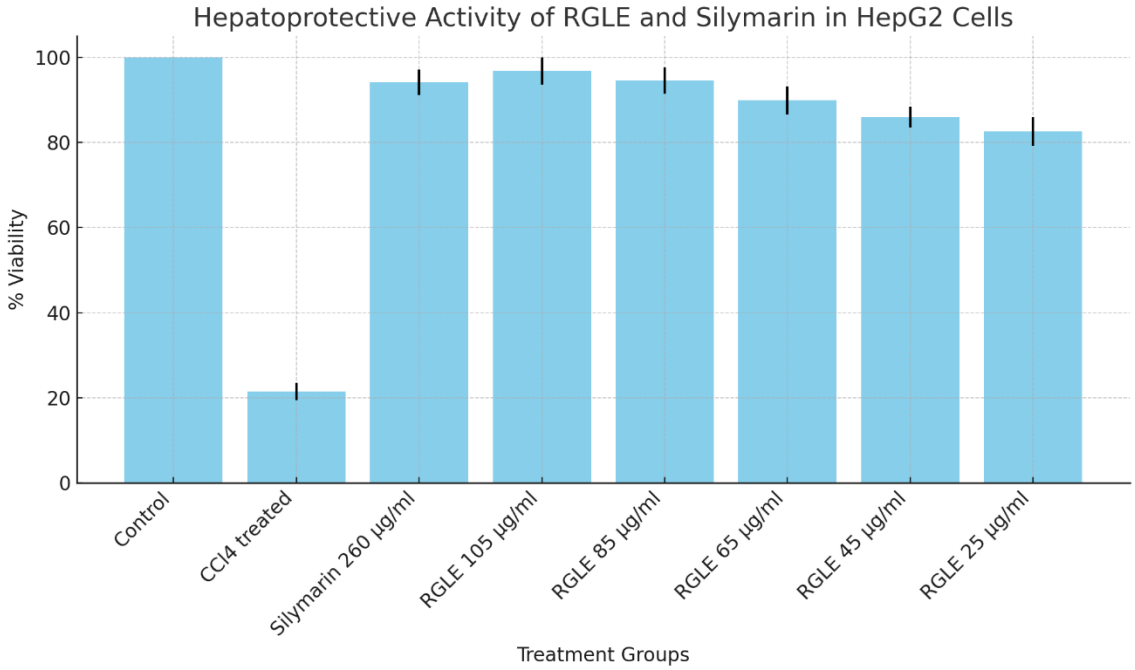

Figure 3. HepG2 cells that have been exposed to CCl4 exhibit hepatoprotective effects from the RGLE.

4. Conclusion

This present study clearly demonstrated the antiradical and hepatoprotective activity of the methanol extracts of leaves of *Ruta graveolens* (RGLE) in various mechanistic in vitro experimental models. The results revealed that the herbal extract possessed the ability to scavenge or neutralize DPPH radicals indicating its potential role in diminishing oxidative stress caused by free radicals. Moreover, the extract demonstrated potential liver protective efficacy indicating it antiradical role in alleviating liver damage caused by toxicants.

5. Reference

1. Wolf PL. Biochemical diagnosis of liver diseases. Indian Journal of Clinical Biochemistry. 1999;14:59-90.
2. Kataki MS, Kakoti BB, Bhuyan B, Rajkumari A, Rajak PJCjonm. Garden rue inhibits the arachidonic acid pathway, scavenges free radicals, and elevates FRAP: role in inflammation. 2014;12(3):172-9.
3. Subramoniam A, Pushpangadan P. Development of phytomedicines for liver disease. Indian J Pharmacol. 1999;31:166-75.
4. Oh TY, Lee JS, Ahn BO, Cho H, Kim WB, Kim YB, et al. Oxidative damages are critical in pathogenesis of reflux esophagitis: implication of antioxidants in its treatment. Free Radical Biology and Medicine. 2001;30:905-15.
5. S Kataki M, B Kakoti BJCTM. Women's Ginseng (Angelica sinensis): an ethnopharmacological dossier. 2015;1(1):26-40.
6. Kataki MS. Study of Indigenous Plants for The Development of a Hepatoprotective Herbal Formulation and Improvement of Bioavailability Through In Vitro In vivo Experiments.
7. Sarma Kataki M, Murugamani V, Rajkumari A, Singh Mehra P, Awasthi D, Shankar Yadav RJPC. Antioxidant, hepatoprotective, and anthelmintic activities of methanol extract of Urtica dioica L. leaves. 2012;3(1).
8. Gülçin I, Oktay M, Küfrevioglu ÖI, Aslan A. Determination of antioxidant activity of *lichen Cetraria islandica* (L) Ach. Journal of Ethnopharmacology. 2002;79(3):325-9.
9. Masuda Y. [Learning toxicology from carbon tetrachloride-induced hepatotoxicity]. Yakugaku Zasshi. 2006;126(10):885-99.
10. Luper S. A review of plants used in the treatment of liver disease: part two. Alternative medicine review : a journal of clinical therapeutic. 1999;4(3):178-88.
11. Murtaza G, Ullah N, Mukhtar F, Nawazish S, Muneer S, Mariam. Phytotherapeutics: The Emerging Role of Intestinal and Hepatocellular Transporters in Drug Interactions with Botanical Supplements. 2017;22(10):1699.
12. Ellnain-Wojtaszek M, Kruczynski Z, Kasprzak J. Investigation of the free radical scavenging activity of *Ginkgo biloba* L. leaves. Fitoterapia. 2003;74(1-2):1-6.
13. Halliwell B, Gutleridge M. Review article. Oxygen toxicity, oxygen radicals, transition metals and disease Biochemical Journal. 1984;219:1-4.
14. Harborne JB. Phytochemical methods: A guide to Modern Techniques of Analysis. London: Chapman and Hall Publishers; 1973.
15. Slinkard K, Singleton VL. Total Phenol analysis: Automation and comparison with manual methods. American Journal of Enology and Viticulture. 1977;28:49-55.
16. Shimada K, Fujikawa K, Yahara K, Nakamura T. Antioxidative properties of xanthin on autoxidation of soybean oil in cyclodextrin emulsion. Journal of Agricultural and Food Chemistry. 1992;40:945-8.
17. Arzumanian VA, Kiseleva OI, Poverennaya EV. The Curious Case of the HepG2 Cell Line: 40 Years of Expertise. International journal of molecular sciences. 2021;22(23):13135.
18. Zhu L, Zhang Y, Li Y, Wang H, Shen G, Wang Z. Inhibitory effect of lingonberry extract on HepG2 cell proliferation, apoptosis, migration, and invasion. PloS one. 2022;17(7):e0270677-e.
19. Thabrew MI, Hughes RD, McFarlane IG. Screening of hepatoprotective plant components using a HepG2 cell cytotoxicity assay. The Journal of pharmacy and pharmacology. 1997;49(11):1132-5.

20. Hu K, Kobayashi H, Dong A, Jing Y, Iwasaki S, Yao X. Antineoplastic agents. III: Steroidal glycosides from Solanum nigrum. Planta medica. 1999;65(1):35-8.
21. Jainu M, Devi CSS. In Vitro. and In Vivo. Evaluation of Free-Radical Scavenging Potential of Cissus quadrangularis. Pharmaceutical Biology. 2005;43(9):773-9.