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Optimization and Hepatoprotective Activity of Herbal Formulation of Methanolic Extracts of Ruta Graveolens and Angelica Sinensis

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Abstract

The goal of this study was to evaluate the antioxidant and hepatoprotective effects of RAAS, a specially created herbal mixture that contains methanol extracts of Ruta graveolens and Angelica sinensis. A range of in vitro tests were performed as part of the study to assess any potential health advantages of RAAS. The laboratory assays employed to assess the antioxidant capacity of RAAS included the total antioxidant activity assay, the reducing power assay, and the DPPH radical scavenging assay. These evaluations showed that RAAS has strong antioxidant characteristics, demonstrating its capacity to counteract oxidative stress and disarm dangerous free radicals. Additionally, the study examined the hepatoprotective effects of RAAS utilizing a cellular model that included $HepG_2$ cells and carbon tetrachloride-induced liver injury (CCl_4). According to the findings, RAAS significantly protected $HepG_2$ cells from the negative effects of CCl_4-induced liver damage. In conclusion, this study highlights the prospective antioxidant and hepatoprotective properties of RAAS, an herbal formula made from methanol extracts of Ruta graveolens and Angelica sinensis. Strong in vitro antioxidant activity and the capacity to prevent CCl_4-induced damage to liver cells show the potential therapeutic utility of RAAS in reducing oxidative stress and liver-related diseases. Deeper understanding of its uses in boosting liver health and general wellbeing may

CC License CC-BY-NC-SA 4.0	*come from additional studies in the clinical setting and other fields of study.* ***Keywords:*** *Antioxidant, Hepatoprotective, Ruta graveolens, Angelica sinensis, Herbal formula*

1. Introduction

The liver or the hepatic system is the primary regulatory structure in charge of the synthesis and production of numerous chemicals and enzymes as well as the metabolism of all xenobiotics. There is always evidence that several liver metabolic processes and functions are disrupted when there are hepatic parenchymal damage [1]. Hepatotoxicity and liver damage are caused by a wide variety of aetiologic modalities, including various mediators of infections, multiple biochemical moieties, viruses & bacteria, and environmental pollutants. The expanding volume of published literature suggests that free radicals and oxidative stress may significantly contribute to the pathogenesis of many liver disorders. Numerous experimental hepatotoxins, including carbon tetrachloride, paracetamol, and others, have been connected to the production of free radicals and oxidative stress, which have now been shown to be the processes by which these toxins injure the liver [1, 2]. These pathways, along with hepatic fibrosis, liver damage, necrosis, and apoptosis, became the pathophysiological basis of many liver illnesses [2].

In order to maintain a protective position in the physiology, enzymes including catalase (CAT), glutathione peroxidase (GSH), and superoxide dismutase (SOD) are periodically used as part of the physiological antioxidant defense system to neutralize free radicals. Therefore, any disruption of this enzyme system made the physiological system more vulnerable to harm. Additionally, the main culprit for causing oxidative stress is highlighted as vitamin deficiency together with an influx of free radicals and a decreased amount of the aforementioned enzymes [3, 4]. Taking into account all of these ideas, current research is increasingly focusing on the neutralization of free radicals, the prevention of lipid peroxidation, and the restoration of the antioxidant enzyme system, and these modalities have been criticized as the likely treatment and prevention goals of hepatotoxicity and other liver ailments [5, 6].

Free radicals are produced through regular metabolic activities of metabolic pathways and can also be acquired from the environment. Unpaired electrons can be found in free radicals. Reactive oxygen species (ROS), such as hydroxyl radicals, superoxide radicals, and reactive nitrogen species (RNS) are some examples of these. But these extra free radicals generated by endogenous or exogenous sources result in oxidative damage to a number of biological components, such as proteins, lipids, nucleic materials & acids and other macromolecules. They peroxidize membrane lipids, a telltale indicator of inflammation, decrease membrane fluidity, decrease enzyme and receptor activity, damage membrane protein, and damage DNA by destroying the unsaturated fatty acids in the biomembranes.
According to research, one of the primary factors that contribute to the onset of many chronic and degenerative diseases, such as ischemic heart disease, atherosclerosis, diabetes mellitus, ageing, immunosuppression, neurodegenerative diseases, and cancer, is oxidative stress brought on by free radicals. These elements contribute to the onset of several degenerative diseases as well as the quickening of ageing [7, 8]. The natural antioxidants are the main line of defense against damage caused by free radicals. By giving free radicals an electron to stabilize and balance, antioxidants stop them from causing harm to the proteins, DNA, macromolecules and cells. Through a number of potential mechanisms, including direct

interaction with and scavenging of free radicals, reduction of peroxides, chelation of transition metals, and stimulation of the antioxidative enzyme defense system as part of immune system's defense arsenal, antioxidants are thought to have positive effects on promoting health. Antioxidants can thus be utilized to counteract the negative and pathogenic effects of free radicals and restore the body's physiological systems to their natural state. The antioxidants in use are either produced synthetically or organically from plants. Despite the fact that there are a number of synthetic antioxidants, such butylated hydroxyl anisole (BHA) and butylated hydroxyl toluene (BHT), they are extremely risky and have been proven to be harmful and deteriorating. Consumers trust natural antioxidants because they believe they are secure. Natural antioxidants that are both efficient and abundant include ascorbic acid, carotenoids, and phenolic substances. Due to the existence of antioxidant components such phenolics, proanthocyanidins, and flavonoids, plants have been shown to have antioxidant activity.

An imbalance between the body's ability to eliminate ROS with antioxidant system and the generation of ROS leads to oxidative stress, a physiological condition. Oxidative stress affects the liver very adversely as it plays a key role in metabolic and detoxifying activities. When ROS levels get too high, they can seriously harm liver cells and result in conditions including cirrhosis, fatty liver disease, hepatitis, and even hepatocellular cancer. CAT, SOD, and GSH are a few of the antioxidant defense systems the liver is endowed with to combat oxidative stress. The liver, however, may be harmed if these defenses are overpowered. This harm may cause fibrosis, inflammation, and reduced liver function, all of which may have harmful effects on one's health.

Since ancient times, medicinal herbs have been valued for their therapeutic potential, and they are essential in preventing oxidative stress and liver damage. Many of these plants include bioactive substances such polyphenols, flavonoids, and saponins, which have potent antioxidant activities. Green tea, milk thistle (*Silybum marianum*), and turmeric (*Curcuma longa*) and tea (*Camellia sinensis*) are a few well-known examples [9, 10]. These substances from plants can lessen oxidative stress in the liver and counteract ROS. For instance, silymarin, a flavonoid compound recognized for its hepatoprotective effects, is present in milk thistle.

By boosting the liver's built-in antioxidant defences, encouraging tissue regeneration, and lowering inflammation, it aids. Curcumin, an anti-inflammatory and antioxidant compound found in turmeric, helps reduce liver damage and prevent fibrosis. Green tea polyphenols, in particular epigallocatechin gallate (EGCG), have promise for shielding the liver from damage brought on by oxidative stress. A proactive method to protect liver health is to include medicinal herbs in one's diet or to take them as supplements. However, before using herbal medicines, it's crucial to speak with a medical practitioner because they may interact with certain prescriptions and cause negative effects. It appears that liver damage and a number of liver illnesses are greatly influenced by oxidative stress. Antioxidants and a reduction in oxidative stress are provided by medicinal herbs, which provide a safe and efficient method of liver protection. These herbal medicines have a crucial role to play in promoting liver health and preventing liver damage when taken sensibly and with expert direction [7, 8, 11].

Ruta graveolens, popularly known as rue, has an extended history of utilization as medicine. This fragrant plant has a position in many different traditional medical practices across the world. It is prized for its many medicinal qualities, which include anti-inflammatory actions that are used to treat ailments including arthritis and joint pain. Rue is well known for its

digestive benefits, including its ability to reduce gas, bloating, and flatulence. It is advantageous for lowering muscle spasms and menstrual cramps due to its antispasmodic qualities. Rue has been used in several cultures to treat migraines. Rue also includes substances with antioxidant capabilities that provide defense against oxidative stress. Although it has shown antibacterial promise for healing wounds and skin infections, its use needs to be handled carefully because excessive use can be harmful. Before adding rue to one's health regimen, it is recommended to speak with a healthcare provider or herbalist. Due to potential hazards, pregnant or nursing women should avoid using rue [12].

The herb *Angelica sinensis*, also called Dong Quai or "female ginseng," is venerated in traditional Chinese medicine and worldwide for its many therapeutic uses. It is primarily praised for its contribution to the health of women; uses include controlling menstrual cycles, easing menstrual cramps, and treating menopausal symptoms. Its phytochemical components are thought to support hormonal harmony. Dong Quai is a beneficial treatment for ailments like anaemia and poor circulation since it has the ability to improve blood circulation. It also has anti-inflammatory effects, which are used to treat inflammation and joint discomfort. The herb *Angelica sinensis*, also called Dong Quai or "female ginseng," is venerated in traditional Chinese medicine and worldwide for its many therapeutic uses. It is primarily praised for its contribution to the health of women; uses include controlling menstrual cycles, easing menstrual cramps, and treating menopausal symptoms. Its phytochemical components are thought to support hormonal harmony. Dong Quai is a beneficial treatment for ailments like anaemia and poor circulation since it has the ability to improve blood circulation. It also has anti-inflammatory effects, which are used to treat inflammation and joint discomfort [11].

In summation, another issue or gap that emerged from the literature pointed to a lack of appropriate hepatoprotective management or therapy options and also suggested that the current hepatoprotectives had highly negative side effects [13]. Therefore, the latest research activities in the sector have focused on finding a new hepatoprotective form of natural origin that has improved safety characteristics while avoiding any noticeable adverse effects. Natural antioxidants are becoming more important in the fight against chemically or drug-induced toxicity. In response to a need and growing worries, our pharmacological lab created and improved an herbal blend made up of a combination of methanol extracts from the two separate native plants, *Ruta graveolens* and *Angelica sinensis*. The goal of the current investigation was to examine the pharmacologic effects of the improved herbal formula, known as RAAS, on both antioxidant and hepatoprotective efficacy in diverse *in vitro* settings.

2. Material and Methods

2.1. Drugs, Chemicals and Reagents

M Sea Pharmaceuticals in Paonta Sahib, Himachal Pradesh, India provided Silymarin and all the standard drugs as complimentary sample. Other chemicals, drugs and reagents were purchased from Lab India in Delhi, India, and Sigma Chemicals Company in St. Louis, Missouri, respectively. The remaining chemicals and reagents were all analytical-grade and sourced from reliable vendors.

2.2. Preparation of Extracts

Angelica sinensis and *Ruta graveolens* were bought from the Herbal Waves in Himachal Pradesh, India. Prof. S. Sharma, Botanist, Himachal Pradesh, India, verified its authenticity. A sample for each plant was delivered to the same institute's herbarium. An electric grinder was used to powder the fresh, air-dried whole plant. Following a five-day soak in methanol

with periodic shaking of the powder, the solvent was filtered. The filtrate was gathered, and the Soxhlet apparatus was used to extract it. The extract was then kept at -4 ^{o}C for later use after being vacuum dried (yield: 18.9%). Finally, the herbal combination codenamed as RAAS was created by combining both extracts in a 1:1 ratio.

2.3. *In Vitro* Assessment of Antioxidant Properties

2.3.1. Total Antioxidant Activity

The previously described thiocyanate technique was used to measure the test sample's total antioxidant activity [14, 15]. 15 mL of water were used to dissolve the test sample (10 mg). 3.6 mL of emulsion of Linoleic acid made in 0.05 M potassium phosphate buffer (pH 7.2) was combined with several concentrations of RAAS as low as 60 µg/mL and as high as 270 µg/mL or 0.06 M of standard compound (pH 7.2). Linoleic acid is emulsified in five millilitres with 18.2 g of Tween-80, 16.8 µl of linoleic acid, and 0.06 M potassium phosphate buffer (pH 7.2). In contrast, the 6.0 mL control contains 3.6 mL of a potassium phosphate buffer and 3.6 mL of an emulsion of linoleic acid (0.05 M, pH 7.2). In a glass flask, the mixture was kept at 37°C while being kept in the dark. Following the $FeCl_2$ reaction and thiocyanate at several points during incubation period, the mix was agitated for 3 min, and the peroxide value was then calculated by reading the absorbance at 510 nm in a spectrophotometer (Shimadzu). Throughout the linoleic acid oxidation, peroxides were produced. These substances transform Fe^{2+} into Fe^{3+}. SCN, whose highest absorbance was at 500 nm, forms compounds with the latter Fe^{3+} ions. Thus, a strong absorption signalled a significant level of linoleic acid oxidation. The solutions devoid of standards or test samples served as a blank. The data on total antioxidant activity is the average of three different analyses. The lipid peroxidation inhibition % was calculated using the formula below:

$$\text{Inhibition (\%)} = (A_0 - A_t / A_0) \times 100$$

Where A_t denoted the optical density of the sample and A_0 the optical density of the control reaction. Each test was run three times, and the mean ± SD values were used to plot the graph. α-Tocopherol was used as a standard antioxidant substance.

2.3.2. Reducing Power

The previously mentioned method was used to analyse the test sample's reducing power [16]. Potassium ferricyanide [$K_3Fe(CN)_6$] was united with buffer phosphate [pH 6.7, 3.6 mL, 0.3 M] and the test sample (50–250 µg/Ml) in 1 mL of distilled water (3.6 mL, 1.5 percent). For 30 minutes, the mixed solution was incubated at 55 °C. The blend was given a portion (3.6 mL) of trichloroacetic acid (12%). After that, the mixture was centrifuged at 4000 rpm for 12 minutes. 3.6 mL of the solution's supernatant layer, 3.6 mL of distilled water, and 3.6 mL of $FeCl_3$ were combined (0.6 mL, 0.2 percent). At 705 nm, the absorbance was measured using a spectrophotometer (Shimadzu). Greater reducing power was suggested by the reaction mixture's higher absorbance. α-Tocopherol was used as a standard antioxidant substance.

2.3.3. Determination of DPPH (1-1-Diphenyl- 2-Picryl Hydrazyl) Radical Scavenging Activity

DPPH was used to determine the test sample's capacity to scavenge free radicals, using the previously described procedure [17]. Three millilitres of test sample solution in water were mixed with varied quantities of DPPH (ethanol solution of 0.2 mM solution of DPPH) (10-250 µg/mL). The mixture was vigorously stirred, then left to stand for 35 minutes at room temperature. A spectrophotometer was then used to measure the absorbance at 518 nm (Shimadzu). Lower absorbance in a reaction mixture meant that it was better able to scavenge free radicals. The following equation was used to compute the % DPPH scavenging effect**:**

The % scavenging effect of $DPPH^{\bullet} = [(A_x - A_y / A_x) \times 100]$
Where A_y denoted the optical density of the reference sample (BHA) or test sample (RAAS) and A_x denoted the optical density of the control reaction. Each test was run three times, and the mean ± SD values were used to plot the graph. BHA was the standard antioxidant substance.

2.4. *In Vitro* Appraisal of Hepatoprotective Action

Hepatic ($HepG_2$) Cell Line was used to assess the in vitro hepatoprotective efficacy in a CCl_4-induced hepatotoxicity paradigm.

2.4.1. Cell line

$HepG_2$ is a extensively investigated liver cell line originated from human that has contributed significantly to our knowledge of drug metabolism, liver biology, and a variety of disorders associated with the liver. These cells are an important resource for *in vitro* study since they were originally isolated from a liver tumor of a 15-year-old Caucasian guy in 1970 [18]. For numerous reasons, $HepG_2$ cells are especially valuable. The main functioning cells in the liver, mature hepatocytes, can be distinguished by the traits these cells display. The production of proteins, the breakdown of lipids, and the metabolism of drugs are all tasks that these cells are capable of performing. $HepG_2$ cells are used by researchers to look into processes unique to the liver, including as drug toxicity, hepatitis virus replication, and the effects of various substances on liver health. Additionally, $HepG_2$ cells may be grown and maintained in a variety of labs and research facilities due to their simplicity. They provide a reliable and repeatable model for investigating processes related to the liver and can be utilized to test potential medication candidates for toxicity or impacts on liver function. In inference, the $HepG_2$ cell line represents an essential resource for biomedical research, particularly in the disciplines of hepatology, toxicology, and drug discovery. Its liver-specific features and simplicity of use have made it an invaluable tool for learning about liver biology and expanding our understanding of disorders associated with the liver and how drugs affect them [18, 19].

2.4.2. Hepatoprotective Activity in $Hepg_2$ Cell Line

Based on the protection of human liver-derived HepG2 cells against CCl_4-induced damage, hepatoprotective efficacy was screened [20] and determined by assessing mitochondrial synthesis employing the tetrazolium assay [21]. $HepG_2$ cells were routinely multiplied and sub-cultured as monolayers in DMEM supplemented with 10% newborn calf serum. The procedures in this investigation used cells that had previously undergone a 10-day batch culture. The cells were then removed, plated on 96-well Nunclon microtitre plates at a density of 27,000 cells per well, and left to repose for 24 hours at 37°C in a humid environment containing 6% carbon monoxide. Following that, the cells were subjected to a toxicant (medium containing 1% CCl_4) together with varying concentrations of the total alkaloid fraction, the medium alone, or both (as normal) [20]. Silymarin dosages larger than 250 µg/ml and RAAS dosages greater than 100 µg/ml have both been demonstrated to be harmful to cells, 20 to 100 µg/ml concentrations, or dosages range were utilised. At the conclusion of the experiment, cytotoxicity was determined by determining the $HepG_2$ cells' viability using the MTT reduction assay [21]. The test solution from each well was removed after an hour of incubation and replaced with 60 µl of MTT made in MEM without phenol red. The plates received a light shaking before being incubated for 3.5 hours at 37°C in a 5 percent CO_2 humid atmosphere. The generated formazan was solubilized by removing the supernatant, adding 50 µl of propanol, and gently shaking the plates. Using a microplate reader, the absorbance was calculated at 540 nm.

2.5. Data Analysis and Statistical Treatments

One-way analysis of variance (ANOVA) was used for the statistical analysis, and Turkey was used as a *post hoc* test. The values are shown as mean ± SD. Turkey's Multiple Comparison Test was used to compare the mean values of various groups treated with various dose levels and a positive control group with the normal. $P < 0.05$ was considered significant. GraphPad Prism (Version 7, GraphPad Software, USA) was used for all of the analyses.

3. Results

3.1. *In Vitro* Evaluation of Antioxidant Activity

3.1.1. Appraising the Total Antioxidant Activity

The thiocyanate method is a widely employed approach for assessing the overall antioxidant potential of the tested substance, referred to as RAAS. Under the current experimental conditions, the test sample exhibited robust and efficacious antioxidant capabilities when administered at a concentration of 250 µg/mL. As illustrated in Figure 1, this concentration of the test sample had a notable impact on the peroxidation process of a linoleic acid emulsion. Initially, the antioxidant activity of the test sample was observed to rise in tandem with an increase in incubation time. However, it subsequently exhibited a decline in activity as the incubation time continued to increase. It is worth noting that, despite the test sample's concentration surpassing that of α-tocopherol at 250 µg/mL, its antioxidant efficacy was found to be inferior to that of BHA (Butylated hydroxyanisole) at the same concentration. The percentage inhibition of peroxidation of RAAS, α-Tocopherol and BHA in linoleic acid system at 12 hr was found to be 53.95 ± 1.44, 87.20 ± 2.57 and 22.85 ± 1.66 %, respectively.

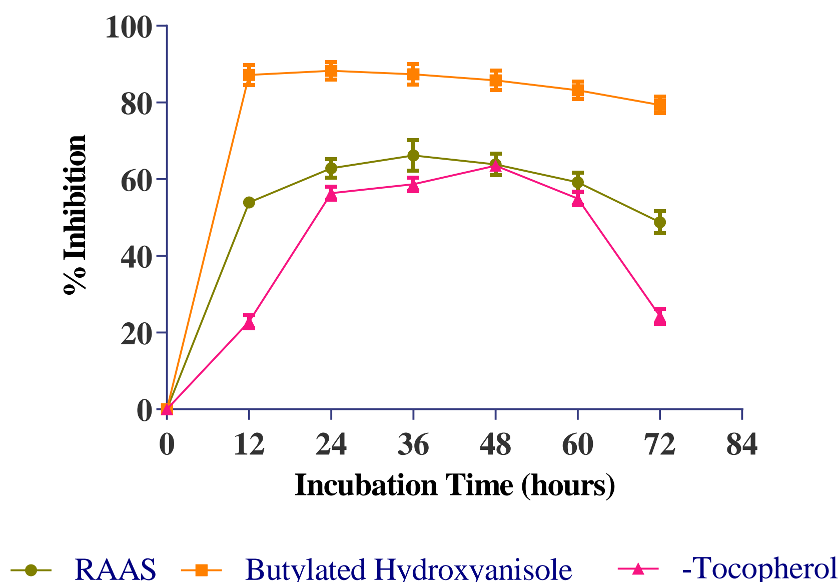

Figure 1. It depicts the total antioxidant activity of RAAS.

3.1.2. Assay of Reducing Power

The test sample, RAAS, had its reducing power assay findings compared to BHT and α-Tocopherol in Figure 2. In the reducing power assay, the $Fe3+$ - $Fe2+$ transition was investigated in the presence of test compounds using the Oyaizu method (Oyaizu, 1986). It was shown that the decreasing power of the test sample rose linearly with test sample concentration. The test samples displayed lesser reductive capability than BHT but stronger

reducing power than α-Tocopherol at all dosages. It was found that the reduction power of RAAS is comparable. The reducing power of the test sample and the standard compounds dropped in the following order: RAAS, α-tocopherol, and BHT [16]. The test samples' concentration demonstrated an enhancement in their reducing capacity. These test samples exhibited a more potent reducing power when compared to α-Tocopherol, but their reductive ability was slightly lower than that of BHT across all dosage levels. Interestingly, the RAAS displayed a similar reducing power as well. The test sample's and the reference compounds' reducing power declined in the following order: BHT, RAAS, and α-tocopherol.

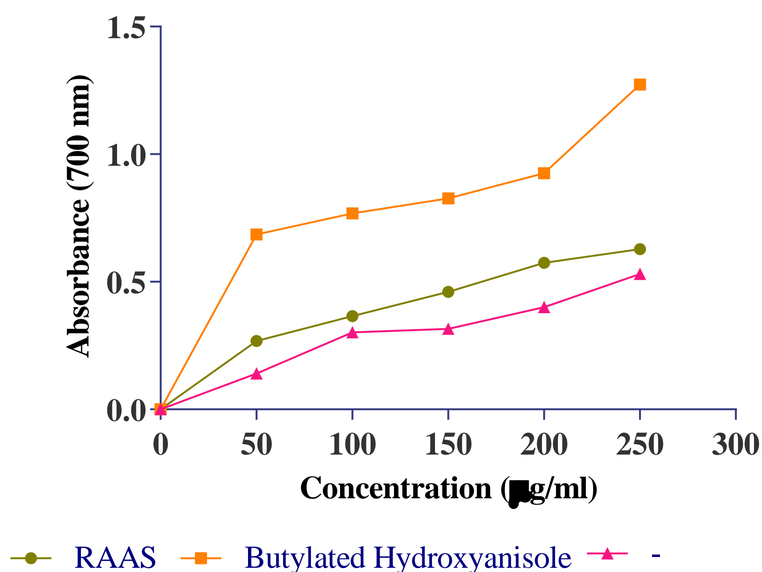

Figure 2. It shows the RAAS's reducing power.

3.1.3. Determination of DPPH (1-1-Diphenyl- 2-Picryl Hydrazyl) Radical Scavenging Activity

A quicker and efficacious in vitro approach to assess antioxidant activity involves the examination of DPPH radical scavenging capability. Comparatively, measuring the DPPH radical's stability when neutralized is a widely adopted method for evaluating antioxidant effectiveness. In order to create a stable diamagnetic molecule, DPPH can accept either an electron or a hydrogen radical. By scavenging DPPH radicals, antioxidants cause a drop in absorbance at 517 nm. In order to evaluate the antioxidant potential of various substances, whether synthetic or natural, DPPH is frequently used as a substrate. BHT was used in this work as a typical radical scavenger. Figure 3 shows the decrease in DPPH radical concentration brought on by the test samples' (RAAS) and the reference substance's (BHT) scavenging capacities at varied doses (range from 10 to 250 µg/mL). The test samples' (RAAS) DPPH radical scavenging capacity was found to be marginally inferior to that of BHT. The test samples (RAAS) showed a 92.95±1.90 percent DPPH scavenging effect at a concentration level of 250 µg/mL, whereas the standard (BHT) showed a 97.11±1.087 percent DPPH scavenging effect at the same concentration. According to the findings, RAAS is an equally effective DPPH radical scavenger to traditional BHT. The equation produced from the linear regression analysis was used to calculate the IC_{50} values for the RAAS and BHT. The estimated IC_{50} values for the reference substance (BHT) and the RAAS were discovered to be 72.12 and 76.18 µg/ml, correspondingly. When the body's ability to combat free radicals is outpaced, oxidative stress results. It is the basic foundation of chronic conditions [22]. The results of this investigation showed that RAAS was a powerful free

radical scavenger that can lessen or even undo the harm that free radicals do to the human body.

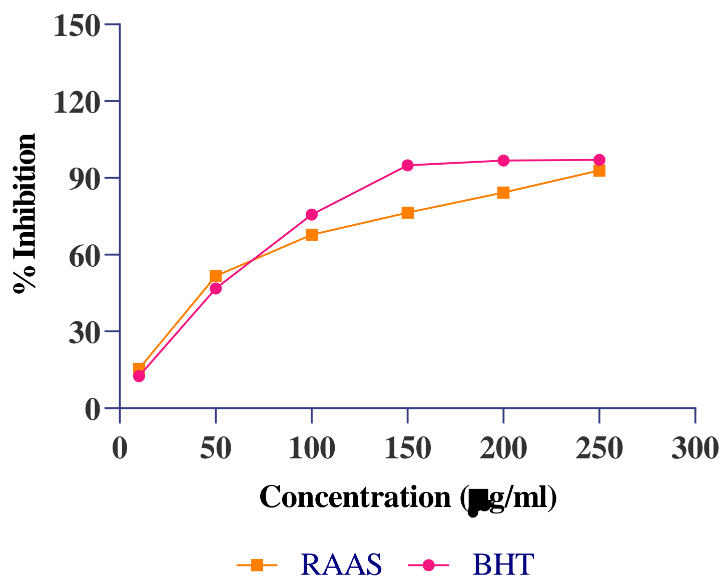

Figure 3. Depicts the ability to DPPH scavenging RAAS

3.2. *In Vitro* Hepatoprotective Activity

3.2.1. Appraising the Hepatoprotective Action In Hepg$_2$ Cells

The HepG$_2$ cells' viability following exposure to CCl_4 was recorded at 19.57 percent. These findings were statistically significant (Compared to the CCl_4-intoxicated group, $P < 0.001$). Notably, the intoxicated cells exhibited a concentration-dependent upsurge in viability when subjected to various concentrations of RAAS. At concentrations of RAAS ranging from 20 to 100 µg/mL, the viability ranged from 80.07±3.75 to 94.44±3.30 percent, as detailed in Table 1. Additionally, when HepG$_2$ cells were treated with RAAS at 80 and 100 µg/mL and compared to the standard Silymarin treatment at 250 µg/mL, the rise in percentage vitality was noticeably significant ($P<0.01$), demonstrating the better potency of RAAS in enhancing cell viability.

Table 1. The RAAS 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	-	19.57 ± 2.25^{x}
CCl4 (1%) + standard silymarin treated	250	92.06 ± 3.32^{y}
CCl4 (1%) + RAAS treated	100	94.44 ± 3.30^{y}
	80	92.07 ± 3.49^{y}
	60	87.24 ± 3.56^{y}
	40	83.35 ± 2.51^{y}
	20	80.07 ± 3.75^{y}

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

4. Discussion

A well-known hazardous substance called carbon tetrachloride (CCl_4) has been utilized a lot in lab settings to harm the livers of experimental mice. Due to its severe toxicity, this substance is now hardly ever found in daily life, but it is a significant instrument for studding and comprehending the origins and pathophysiology of liver injury and considering prospective treatments. A lot of research has been done on CCl_4-induced liver injury since it closely resembles some characteristics of human liver disorders. After being injected, CCl_4 is converted by cytochrome P450 enzymes in the liver into extremely reactive free radicals such trichloromethyl radicals (•CCl_3), which can start a chain reaction of harmful events. In the liver cells, these free radicals and species instigate peroxidation of lipids, which consequences in oxidative stress. Hepatocytes consequently experience membrane deterioration, mitochondrial malfunction, and cellular necrosis. Given that prolonged exposure to CCl_4 can result in the gradual scarring of liver tissue, this model is particularly helpful for researching liver fibrosis and cirrhosis. As immune cells are drawn to the site of injury, which is another defining feature of liver injury, inflammation is also seen. Researchers examine possible therapeutic medicines and investigate the effectiveness of antioxidants, hepatoprotective medications, and anti-fibrotic substances employing the carbon tetrachloride intoxicated liver damage model. It aids in clarifying the fundamental molecular mechanisms driving liver injury and repair. It is crucial to remember that even though the CCl_4 model is useful, its applicability to clinical liver illnesses may have limitations. Clinical research cannot employ CCl_4 because to its severe toxicity in humans, and different liver illnesses can have distinct mechanisms of liver injury. Therefore, extrapolating results from this model to human circumstances should be done with caution. The conclusion is that the experimental model of carbon tetrachloride-induced liver injury is well-established and offers important insights into the mechanisms of liver injury, inflammation, oxidative stress, and fibrosis. Despite its usefulness in the development of prospective liver disease treatments, it is critical to recognize its shortcomings in simulating the complexity of human liver pathologies and the necessity of performing additional research in settings that are clinically pertinent. In light of this, CCl_4 severely damaged $HepG_2$ cell lines in the current investigation [23-25] and significant dose-dependent hepatoprotective effects of RAAS were seen. Additionally, the outcomes of the assays for reducing power and total antioxidant activity revealed that RAAS exhibited significant antioxidant activity as well as reducing ability. In the DPPH radical scavenging model, RASS also demonstrated significant free radical scavenging activity. Reactive oxygen species, which are created when successive molecular oxygen molecules undergo one-electron reductions, are typically linked with oxidative stress. Iron may have a substantial role in the catalytic production of the lipid peroxidation, protein oxidation, GSH oxidation, and DNA damage that may result from this pathway [26-28]. These markers were thoroughly examined and investigated in the current study considering all these correlations. Numerous studies have shown that oxidative stress is a key pathophysiological factor indicating cellular damage that underlies a wide range of illness disorders [29-31]. The current work adequately shown the hepatoprotective and antioxidant properties of RAAS in liver damage in $HepG_2$ cells. Antioxidant potential is noted in this context as an important criteria indicative of the most probable hepatoprotective mechanism. The RAAS considerably showed DPPH scavenging activity, indicating the RAAS has the ability to neutralize free radicals. CCl_4 model showed that moderate to hepatocellular hydropic degeneration, unadorned necrosis, and fibrosis, leukocyte infiltration symptoms were common liver damage. According to a study using $HepG_2$ cell lines, RAAS

significantly protected the liver at the studied dosages. The herbal blend or formula RAAS is a strong antioxidant and hepatoprotective, according to the findings of this study with genuine and dependable mechanisms.

5. Conclusion

Evidently, the current investigation demonstrated the antioxidant activity and hepatoprotective effects of the herbal supplement known as RAAS. The results of the study clearly demonstrate the considerable hepatoprotective efficacy of the herbal formula - RAAS.

Conflict of Interest

According to the authors, there have no competing interests.

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