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Rumex Napalensis Leaf Water Extract Demonstrated Liver-Protective Properties And Neutralized Oxidative Damage

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ABSTRACT

This present study aimed to evaluate and investigate the antioxidant, free radical scavenging activity and hepatoprotective activity of the cold macerated water extract of leaves of *Rumex napalensis.* This study explored the potential pharmacological properties of *Rumex napalensis* water extract (RN-WE) through a series of in vitro assays. Initially, the extraction process using cold maceration with water yielded a high percentage yield of 9.1%, indicating efficient extraction of bioactive compounds. Phytochemical analysis of RN-WE revealed the presence of phenols, flavonoids, saponins, alkaloids, phytosterols, and anthocyanins, highlighting its diverse chemical composition. Subsequent antioxidant assays demonstrated potent DPPH radical scavenging activity of RN-WE, surpassing the standard antioxidant BHT in certain concentrations, and exhibiting a dose-dependent response. Additionally, the extract exhibited remarkable total antioxidant capacity comparable to or even higher than standard antioxidants BHA and α-Tocopherol, further indicating its strong antioxidant potential. Moreover, RN-WE demonstrated significant hepatoprotective effects against CCl4-induced hepatic injury in HepG2 cells, with higher concentrations showing greater efficacy in improving cell viability. These findings underscored the promising therapeutic potential of RN-WE as a natural antioxidant and hepatoprotective agent, warranting further exploration for its clinical applications in combating oxidative stress-related diseases and liver disorders.

Keywords: *Rumex napalensis*, Antioxidant, Hepatoprotective, CCl_4-induced liver injury, HepG2 cells, DPPH radical, Cold maceration

INTRODUCTION

The liver, the largest internal organ in the human body, plays a vital role in maintaining overall health and well-being. Situated in the upper right portion of the abdomen, the liver is tasked with an array of critical functions essential for metabolic processes, detoxification, and overall homeostasis [1, 2]. One of its primary functions is metabolic regulation, as it serves as the metabolic powerhouse of the body, responsible for processing nutrients, synthesizing proteins, and regulating glucose levels. Through complex biochemical pathways, the liver metabolizes carbohydrates, fats, and proteins obtained from the diet, converting them into energy sources or storing them for future use. Additionally, the liver produces bile, a substance crucial for the digestion and absorption of fats in the small intestine. Beyond its metabolic functions, the liver acts as a central detoxification hub, filtering and removing toxins, drugs, and harmful substances from the bloodstream. Hepatocytes, the main functional cells of the liver, contain specialized enzymes that facilitate the breakdown and elimination of toxins, ensuring that harmful compounds are neutralized and excreted from the body. This detoxification process is essential for maintaining systemic health and preventing the accumulation of harmful substances that could lead to organ damage or dysfunction. Moreover, the liver plays a pivotal role in the immune system, serving as a frontline defense against pathogens and infections. Kupffer cells, specialized immune cells located within the liver sinusoids, help identify and eliminate foreign invaders, such as bacteria and viruses, thereby protecting the body from microbial threats. Additionally, the liver synthesizes immune proteins called acute-phase proteins, which play a crucial role in the body's response to inflammation and infection [3, 4].

Furthermore, the liver is indispensable for maintaining blood homeostasis and regulating various biochemical parameters. It stores essential vitamins and minerals, including vitamin A, vitamin D, vitamin B12, and iron, which are released into the bloodstream as needed to support various physiological functions. The liver also regulates blood clotting by synthesizing clotting factors and fibrinogen, essential components of the coagulation cascade. In assumption, the liver is a multifunctional organ with diverse roles critical for maintaining overall health and vitality. Its metabolic, detoxification, immune, and regulatory functions underscore its importance in ensuring proper physiological balance and supporting the body's essential processes. Understanding the significance of the liver's functions highlights the importance of adopting lifestyle practices that promote liver health, such as maintaining a balanced diet, avoiding excessive alcohol consumption, and staying physically active. Additionally, regular medical check-ups and screenings can help detect and address liver-related issues early, ensuring optimal liver function and overall well-being [4, 5].

Hepatotoxicity, or liver toxicity, refers to the adverse effects on the liver caused by exposure to harmful substances or medications. It is a significant concern worldwide due to the prevalence of liver diseases and the widespread use of pharmaceutical drugs and environmental toxins. Hepatotoxicity can manifest in various forms, ranging from mild liver enzyme elevations to severe liver damage, cirrhosis, or even liver failure. Common culprits of hepatotoxicity include alcohol, certain prescription medications, over-the-counter drugs, herbal supplements, industrial chemicals, and environmental pollutants [6, 7]. The need for alternative therapies, particularly natural medicinal plants, arises from several factors. Firstly, conventional treatments for liver diseases often come with adverse side effects and limited efficacy, necessitating the exploration of safer and more effective alternatives. Natural medicinal plants offer a promising avenue due to their rich phytochemical composition and potential therapeutic properties. Many plants contain

bioactive compounds with hepatoprotective, antioxidant, anti-inflammatory, and detoxifying effects, making them valuable candidates for liver health promotion and disease management [7]. Furthermore, natural medicinal plants have been used for centuries in traditional medicine systems worldwide, with a long history of anecdotal evidence supporting their efficacy and safety. Traditional herbal remedies often leverage the synergistic interactions of multiple plant compounds, enhancing their therapeutic potential and mitigating potential adverse effects. The holistic approach of herbal medicine, which considers the interconnectedness of mind, body, and environment, aligns with the principles of integrative and personalized healthcare, emphasizing the importance of addressing underlying imbalances rather than merely treating symptoms.

Moreover, natural medicinal plants offer a sustainable and environmentally friendly approach to healthcare, promoting biodiversity conservation and reducing reliance on synthetic pharmaceuticals with potentially harmful environmental impacts. By harnessing the healing power of nature, herbal medicine can provide accessible, affordable, and culturally relevant healthcare solutions, particularly in resource-limited settings where access to conventional medical services may be limited. Despite the promising benefits of natural medicinal plants, further research is needed to validate their safety, efficacy, and mechanisms of action through rigorous scientific investigation. Well-designed preclinical studies, clinical trials, and systematic reviews are essential for establishing evidence-based guidelines for the use of herbal remedies in liver health and disease management. Collaborative efforts between traditional healers, scientists, healthcare professionals, and regulatory authorities are crucial for integrating traditional knowledge with modern scientific methodologies, ensuring the responsible use and promotion of natural medicinal plants as alternative therapies for hepatotoxicity and liver diseases. Through interdisciplinary research and evidence-based practice, natural medicinal plants can play a pivotal role in complementing conventional treatments and improving outcomes for individuals affected by liver-related conditions [5, 8, 9]. Therefore, this research work aimed to evaluate the antioxidant, free radical scavenging activity and hepatoprotective activity of the cold macerated leaf extract of *Rumex napalensis* in various mechanistic *in vitro* models.

MATERIAL AND METHODS

Drugs and chemicals

Throughout this study, a variety of chemicals and drugs were employed to conduct the experiments and analyses. Many of these chemicals were obtained from reputable suppliers such as Sigma Aldrich, Himedia, and Lobachem, India. Commonly used chemicals included solvents such as ethanol and water for extraction purposes, as well as reagents and standards for phytochemical screening assays, antioxidant activity assays, and hepatoprotective activity assays. Additionally, specific drugs and compounds were utilized, including Butylated Hydroxytoluene (BHT) as a standard antioxidant, silymarin as a standard hepatoprotective agent, and carbon tetrachloride (CCl_4) for inducing liver injury in HepG2 cells. BHT and silymarin were acquired as gift samples from Resenta, Himachal Pradesh.

Collection and authentication of the medicinal plant

Fresh *Rumex napalensis* leaves were meticulously collected from their natural habitats in the Kullu region of Himachal Pradesh. The selection of plants was guided by a comprehensive approach, incorporating insights from local herbalists who have firsthand experience with traditional medicinal plants, as well as data gathered through an extensive ethnomedical survey. This combined methodology ensured the identification and collection of authentic specimens with

known medicinal properties. To validate the botanical identity of the collected plant material, accredited taxonomists were engaged to examine and confirm their classification. These experts received the plant samples and conducted thorough morphological and taxonomic analyses to verify their identity as *Rumex napalensis*. This rigorous verification process is essential for ensuring the accuracy and reliability of the botanical data associated with the collected specimens. For documentation and future reference, voucher specimens labelled as BKSK-952-2022811 were carefully prepared and preserved. These voucher specimens serve as tangible evidence of the plant's identity and origin, providing a valuable resource for scientific research, conservation efforts, and further studies in ethnobotany and herbal medicine. By maintaining these voucher specimens in a designated repository, their availability for reference and verification purposes is ensured, contributing to the integrity and reproducibility of research findings related to *Rumex napalensis* and its medicinal properties.

Extraction of the medicinal plant

A kilogram of fresh *Rumex napalensis* leaves was meticulously gathered, after which they underwent a careful drying process in the shade. Once fully dried, the leaves were ground into a fine powder using a mechanical grinder, resulting in a 300-gram dry sample ready for further processing. The powdered sample was then subjected to solvent extraction to isolate the bioactive compounds present in the plant material. For this purpose, 300 grams of the dried sample were extracted twice using water as the solvent. Each extraction utilized 1000 millilitres of water at a temperature of 25°C and employed a cold maceration technique, allowing for gentle extraction over a period of 7 days. This method ensures the retention of heat-sensitive compounds and maximizes the extraction efficiency. Following the extraction process, the obtained material underwent concentration using a rotary evaporator operating at 45°C under reduced pressure. This step facilitated the removal of the solvent, resulting in the formation of a concentrated extract. The yield of the extract was determined to be 9.2%, indicating the efficiency of the extraction process in isolating the desired compounds from the plant material. The final product obtained from the water extraction of *Rumex napalensis* leaves was referred to as RN-WE extract. This water extract contains a concentrated mixture of bioactive compounds extracted from the plant material, which may possess various pharmacological and therapeutic properties. The RN-WE extract serves as a valuable resource for further research and experimentation aimed at elucidating the medicinal potential of *Rumex napalensis* and its applications in traditional and modern medicine.

Preliminary phytochemical screening

A comprehensive preliminary phytochemical screening was conducted on the extract using a diverse array of well-established tests, as detailed in prior literature. This systematic screening aimed to elucidate the phytochemical composition of the extracts and the herbal blend, shedding light on the presence of various bioactive compounds with potential pharmacological significance. The screening process involved the application of standard qualitative chemical tests to detect the presence of specific classes of phytochemicals known for their medicinal properties. These tests encompassed a range of analytical techniques tailored to identify different groups of compounds, including alkaloids, flavonoids, phenols, tannins, saponins, terpenoids, and glycosides, among others. Alkaloids, for instance, were detected using methods such as Dragendorff's reagent or Mayer's reagent, which produce characteristic precipitates or color changes in the presence of alkaloidal compounds. Similarly, the presence of flavonoids and phenols was assessed using tests like the Shinoda test or the ferric chloride test, which yield distinctive color changes indicative of

the presence of these compounds. Tannins, another important class of phytochemicals with antioxidant and antimicrobial properties, were identified using methods such as the ferric chloride test or the gelatin test, which produce precipitates or turbidity in the presence of tannins. Saponins, known for their foaming properties and potential therapeutic effects, were detected using foam tests or hemolysis assays. Furthermore, terpenoids, which encompass a diverse group of compounds including essential oils and resinous substances, were identified using specific tests such as the Liebermann–Burchard test or the Salkowski test, which yield characteristic color changes or precipitation reactions in the presence of terpenoid compounds. Additionally, the screening process included tests for glycosides, such as the Bornträger's test or the Keller–Kiliani test, which are designed to detect the presence of glycosidic bonds in plant extracts. By employing this comprehensive battery of tests, the preliminary phytochemical screening provided valuable insights into the chemical composition of the extract. The presence of various phytoconstituents identified through these tests suggests the potential pharmacological diversity and therapeutic utility of the extract, warranting further investigation into their bioactivity and medicinal applications [10].

Antioxidant activity: Role in oxidative stress

Measurement of DPPH scavenging activity

The antioxidant activity of the extract was evaluated by assessing its ability to scavenge 2,2-diphenyl-1-picrylhydrazyl (DPPH) radicals using a modified version of the previously described methodology [11, 12]. In this assay, the working solution of DPPH in ethanol was mixed with varying concentrations of the diluted extract, and the resulting mixtures were incubated for 30 minutes at room temperature in the dark to allow for reaction completion. The absorbance of each mixture was then measured at 517 nm using a spectrophotometer. To initiate the assay, the working solution of DPPH was prepared in ethanol, and aliquots of the diluted extract were added to the DPPH solution at different concentrations. The volume of each mixture was adjusted to 1.0 mL using ethanol as needed. Prior to centrifugation at 3000g for 10 minutes, the mixtures were vigorously vortexed to ensure thorough mixing. Subsequently, the mixtures were allowed to stand at room temperature for 30 minutes in the absence of light to facilitate the reaction between the extract and DPPH radicals. After the incubation period, the mixtures were centrifuged to separate the supernatant from any insoluble components. The absorbance of the resulting supernatants was then measured at 517 nm using a spectrophotometer. It's important to note that, except for the extract, all reagents including DPPH solution and ethanol were included in the control setup. The percentage of DPPH scavenging activity exhibited by the extract was calculated using the following formula:

$$\%\ DPPH\ Scavenging\ activity\ = \frac{A_{control} - A_{Sample}}{A_{Control}} \times 100$$

where A sample and A control represent the absorbance values of the control setup and the sample extract, respectively, measured at 517 nm.

This methodological approach allows for the quantitative assessment of the antioxidant activity of the extract by determining its ability to neutralize DPPH radicals. The higher the percentage of DPPH scavenging activity, the greater the antioxidant potential of the extract. By employing this assay, researchers can gain insights into the antioxidant capacity of medicinal plants and their potential therapeutic applications in combating oxidative stress-related diseases.

Determination of total antioxidant capacity (TAC)

The total antioxidant capacity (TAC) of the extract was assessed using a TAC test kit, which measures the overall antioxidant capacity based on the reaction between Fe^2+ and phenanthroline, with absorbance measured at 520 nm using a spectrophotometer. The results were expressed as units of total antioxidant capacity per gram of extract (U/g). To determine the TAC, the quantity of antioxidants required to increase the absorbance by 0.01 in 1 mL of reaction liquid at 37°C, known as one unit (U), was calculated. The formula used for this calculation is as follows:

$$TAC = \frac{OD_U - OD_C}{0.01 \times 30} \times \frac{V_0}{V_1} \times N$$

where ODU is the absorbance value of the test sample and ODC is the absorbance value of the reagent blank; V0 is the total volume of the reaction liquid in millilitres; V1 is the volume of the extract in millilitres.; N is the fold of dilution of the sample prior to testing; The reaction time is thirty minutes [11, 12]. This formula allow ed for the calculation of the TAC based on the difference in absorbance between the test sample and the reagent blank, normalized to the standard unit of antioxidant capacity (U). The volume of the extract, dilution factor, and reaction time are all accounted for in the calculation to ensure accuracy and consistency in the assessment of antioxidant capacity. By quantifying the total antioxidant capacity of the extract, researchers can evaluate its potential to neutralize free radicals and protect against oxidative stress-related damage. This information is valuable for assessing the overall health-promoting properties of the extract and its potential applications in the prevention and treatment of various diseases associated with oxidative stress.

Hepatoprotective activity in **CCl4 induced injury in HepG2 cells.**

The method for evaluating the hepatotoxicity of RN-WE in a CCl4-induced hepatic damage model using HepG2 cell line involved several key steps [13]. Initially, HepG2 cells were cultured in Dulbecco's Modified Eagle Medium (DMEM) supplemented with fetal bovine serum (FBS) and penicillin-streptomycin solution. These cells were seeded onto appropriate cell culture plates and allowed to adhere overnight to ensure proper cell attachment and growth. Following cell adherence, the cells were divided into different treatment groups, including a control group, a CCl4-induced damage group, and various treatment groups with different concentrations of the extract. The CCl4-induced damage was simulated by exposing the cells to a predetermined concentration of carbon tetrachloride (CCl4) for a specific duration to induce hepatotoxicity. After the induction of hepatic damage, the cells were treated with the extract at different concentrations for a predetermined period. The extract was dissolved in an appropriate solvent (DMSO) to ensure proper delivery to the cells. Control groups received only the vehicle solvent to account for any potential effects of the solvent on cell viability. Subsequently, MTT assay was performed to evaluate the hepatotoxicity and potential protective effects of the extract.

Statistcal Treatments

Each experiment was conducted in triplicate, with three duplicates performed for each run to ensure robustness and reliability of the results. Mean values along with standard deviations (SD) were employed to represent all data points, providing a comprehensive overview of the experimental outcomes. For post-hoc comparisons between different experimental groups, mean values were subjected to statistical analysis using a one-way analysis of variance (ANOVA) followed by Dunnett's multiple comparison test. Statistical significance was established at a threshold of $P<0.05$, indicating that differences between groups were considered significant when the probability of their occurrence by chance alone was less than 5%.

RESULTS AND DISCUSSION

Extraction of the plant

Ground-up, shade-dried leaf powders underwent extraction using a cold maceration process with water as the solvent, a method known for its ability to gently extract bioactive compounds from plant materials while preserving their integrity and potency. This extraction process was carried out over a duration of seven days, allowing sufficient time for the solvents to interact with the plant material and facilitate the extraction of target compounds. Water extraction is well-suited for extracting a wide range of phytochemicals, including polar compounds such as phenolic compounds, flavonoids, and polysaccharides, which are often abundant in plant materials and possess various biological activities. After the extraction period, the resulting extract was obtained with a notable percent yield of 9.1%. This high yield indicates the efficiency of the extraction process in recovering bioactive constituents from the plant material. The extract obtained through this method is expected to contain a diverse array of phytochemicals with potential pharmacological and therapeutic properties. Overall, the cold maceration extraction process using water as the solvent has proven to be an effective and environmentally sustainable method for obtaining bioactive extracts from plant materials. The significant yield obtained underscores the potential of this extraction technique for further applications in research, pharmaceuticals, and natural product development.

Preliminary phytochemical screening

Table 1 presented the results of the phytochemical analysis of the extract RN-WE, revealing the presence or absence of various phytochemical compounds. Phenols, flavonoids, saponins, alkaloids, phytosterols, and anthocyanins were identified in the extract, denoted by a positive symbol (+), indicating their presence. These compounds are known for their diverse pharmacological properties, including antioxidant, anti-inflammatory, antimicrobial, and anticancer activities. Their presence suggests the potential health benefits of the extract, making it a promising candidate for further investigation and potential therapeutic applications. Interestingly, the extract was found to be devoid of fatty acids, as indicated by a negative symbol (-). While fatty acids are essential nutrients and play crucial roles in human health, their absence in the extract may indicate that the extraction process primarily targeted non-lipid components of the plant material. Moreover, the extract tested negative for glycosides based on the Borntrager test. Glycosides are a diverse group of compounds found in plants with various biological activities, including cardioprotective, hypoglycaemic, and anticancer effects. The absence of glycosides in the extract suggests that it may not possess certain bioactivities associated with this class of compounds. Overall, the phytochemical profile of the RN-WE extract indicated its potential as a source of bioactive compounds with therapeutic potential, highlighting the importance of further research to elucidate its pharmacological properties and explore its potential applications in medicine and healthcare.

Table 1. Results indicating presence of phytocompounds in the extract (RN-WE)

Phytochemical Compound Group	RN-WE
Phenols	+
Flavonoids	+
Saponins	+
Alkaloids	+

Fatty Acids	-
Phytosterols	+
Borntrager Test	-
Terpenoids	+
Glycosides	-
Anthocyanins	+

+: Presence, -: Absence

Antioxidant activity

DPPH radical scavenging activity

The results of the DPPH radical scavenging activity assay revealed the antioxidant potential of the RN-WE extract compared to the standard antioxidant, BHT (Butylated Hydroxytoluene), across various concentrations. The DPPH assay is a widely used method to evaluate the ability of compounds to neutralize free radicals, thereby reflecting their antioxidant capacity. At all tested concentrations (10 µg/ml to 500 µg/ml), both BHT and RN-WE exhibited DPPH radical scavenging activity. However, interestingly, the RN-WE extract demonstrated higher scavenging activity compared to BHT at concentrations of 10 µg/ml, 50 µg/ml, and 100 µg/ml. This suggests that the extract possesses potent antioxidant properties, potentially attributed to the presence of bioactive phytochemicals identified in the phytochemical screening. Moreover, the scavenging activity of the RN-WE extract remained consistently high across all tested concentrations, with values ranging from 91.96% to 92.89% at the highest concentration of 500 µg/ml. This indicates that the extract exhibits dose-dependent antioxidant activity, with no significant decrease in efficacy even at higher concentrations. IC50 value was calculated to be −75µg/ml for the extract. The negative value suggested that the IC50 falls outside the range of concentrations tested. It's possible that a higher concentration is needed to achieve 50% scavenging activity. Overall, these results highlight the promising antioxidant potential of the RN-WE extract, suggesting its potential utility as a natural antioxidant agent for combating oxidative stress-related diseases and preserving food and cosmetic products. Further studies, including in vivo experiments and clinical trials, are warranted to fully elucidate the therapeutic applications and mechanisms underlying the antioxidant effects of the RN-WE extract.

Total antioxidant capacity

The table presented data on the total antioxidant capacity (TAC) of the RN-WE extract alongside two standard antioxidants, Butylated Hydroxyanisole (BHA) and α-Tocopherol (Vitamin E), across varying incubation times. Observations revealed fluctuations in TAC values with incubation duration, generally peaking before stabilizing or slightly declining. Interestingly, the TAC values of the RN-WE extract were comparable to, or even higher than, those of the standard antioxidants, underscoring its potent antioxidant activity. This suggested that the extract contained bioactive compounds capable of neutralizing free radicals effectively. Despite occasional lower TAC values compared to the standards, the RN-WE extract consistently demonstrated significant antioxidant capacity, particularly evident at longer incubation times. These findings underscored the extract's potential as a natural antioxidant agent for addressing oxidative stress-related conditions and preserving the stability of various products. Overall, the data highlighted the promising antioxidant properties of the RN-WE extract, warranting further exploration for potential applications in healthcare, food preservation, and cosmetic formulations.

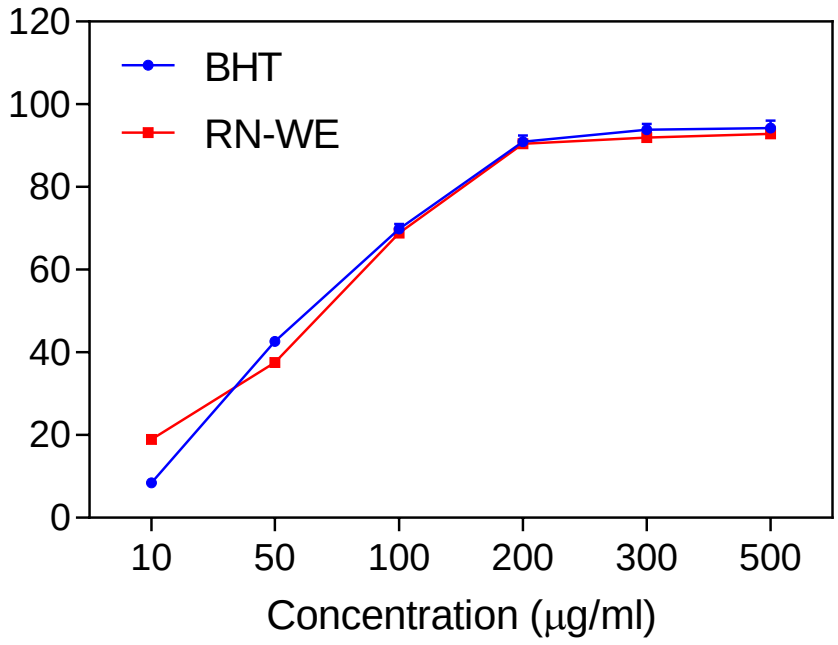

Figure 1. Free radical scavenging activity in terms of DPPH radical scavenging of RN-WE

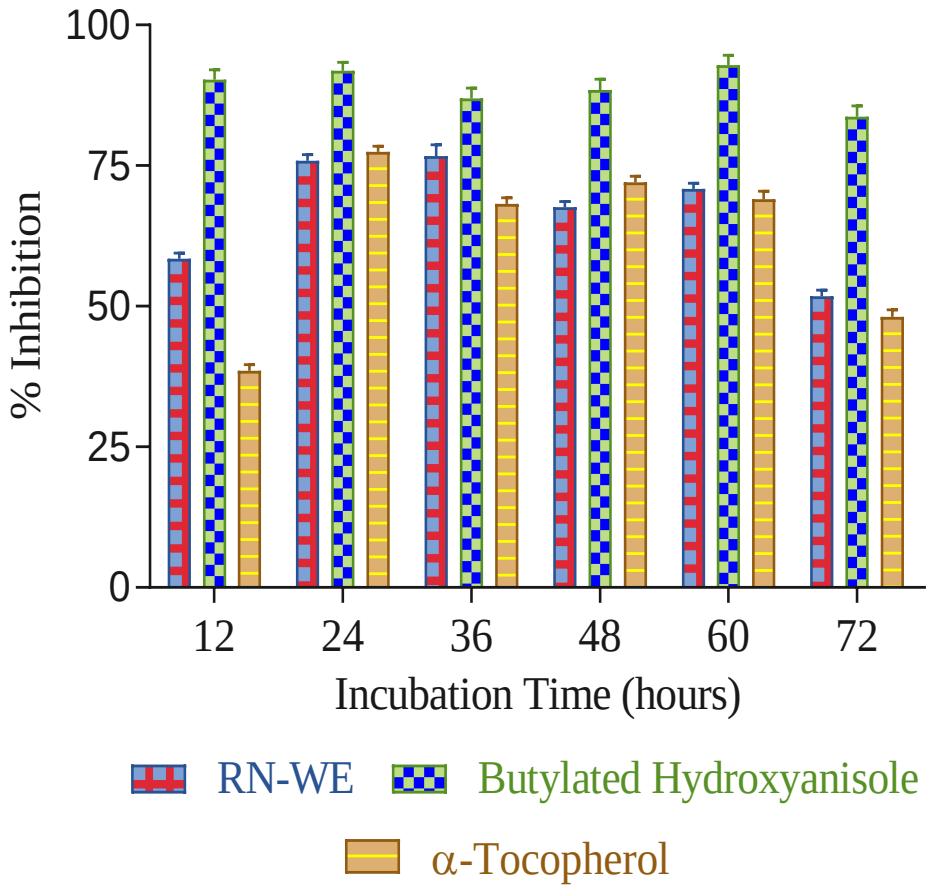

Figure 2. Total antioxidant capacity of the extract RN-WE

Table 2. The calculated values of IC_{50} for DPPH scavenging activity of RN-WE (mean ± SD, n = 3)

Drugs	IC50 (µg/ml)
	DPPH radical
RN-WE	12.56 ± 0.26
Butylated Hydroxy Toluene (BHT)	58.44 ± 1.02

Hepatoprotective activity in **CCl4 induced injury in HepG2 cells**

The table 3 presented the results of the estimation of the hepatoprotective effect of RN-WE in a CCl4-induced injury model using HepG2 cells, with cell viability expressed as a percentage relative to the normal control group. The positive control group, induced with CCl4, exhibited a significantly reduced cell viability of 14.91%, indicating substantial hepatotoxicity compared to the normal control group (100% viability). In contrast, treatment with the standard hepatoprotective agent silymarin at 400 µg/ml resulted in a notable increase in cell viability to 97.78%, demonstrating its effectiveness in protecting against CCl4-induced hepatotoxicity. Similarly, treatment with RN-WE at various concentrations (50-800 µg/ml) showed a dose-dependent increase in cell viability compared to the positive control group. At the highest concentration tested (800 µg/ml), RN-WE exhibited a cell viability of 98.41%, which was statistically significant compared to the positive control group. Additionally, even at lower concentrations (100-400 µg/ml), RN-WE significantly improved cell viability compared to the positive control group. However, at the lowest concentration tested (50 µg/ml), RN-WE showed a slightly lower but still significant cell viability of 80.91% compared to the positive control group. These findings suggest that RN-WE possessed hepatoprotective properties against CCl4-induced hepatic injury in HepG2 cells, with higher concentrations demonstrating greater efficacy. The dose-dependent response observed indicates a potential dose-response relationship, with higher concentrations of RN-WE providing greater protection against hepatotoxicity. Overall, these results support further investigation of RN-WE as a potential therapeutic agent for liver disorders.

Table 3. Estimation of hepatoprotective effect of RN-WE in CCl_4 induced injury in $HepG_2$ cells.

Sample (Treatment Groups)	Concentration (µg/ml)	% Cell Viability
Control (Normal Group)	-	100
Control (Positive Group, CCl_4 induced)	-	14.91 ± 1.08*
Silymarin Treated + CCl_4 (1.5 %) induced (Standard Group)	400	97.78 ± 2.64 #
RN-WE treated + CCl_4 (1.5 %) (Test Group)	800	98.41 ± 3.03 #
	400	95.59 ± 3.01 #
	200	93.68 ± 3.02 #
	100	86.88 ± 3.03 #
	50	80.91 ± 2.10 #
Data were represented as Mean ± SD for 3 replicate determinations as an average. * indicated significant differences compared to normal group ($p < 0.001$); and # indicated significant as compared to Toxicant group (Positive control) ($p < 0.01$).		

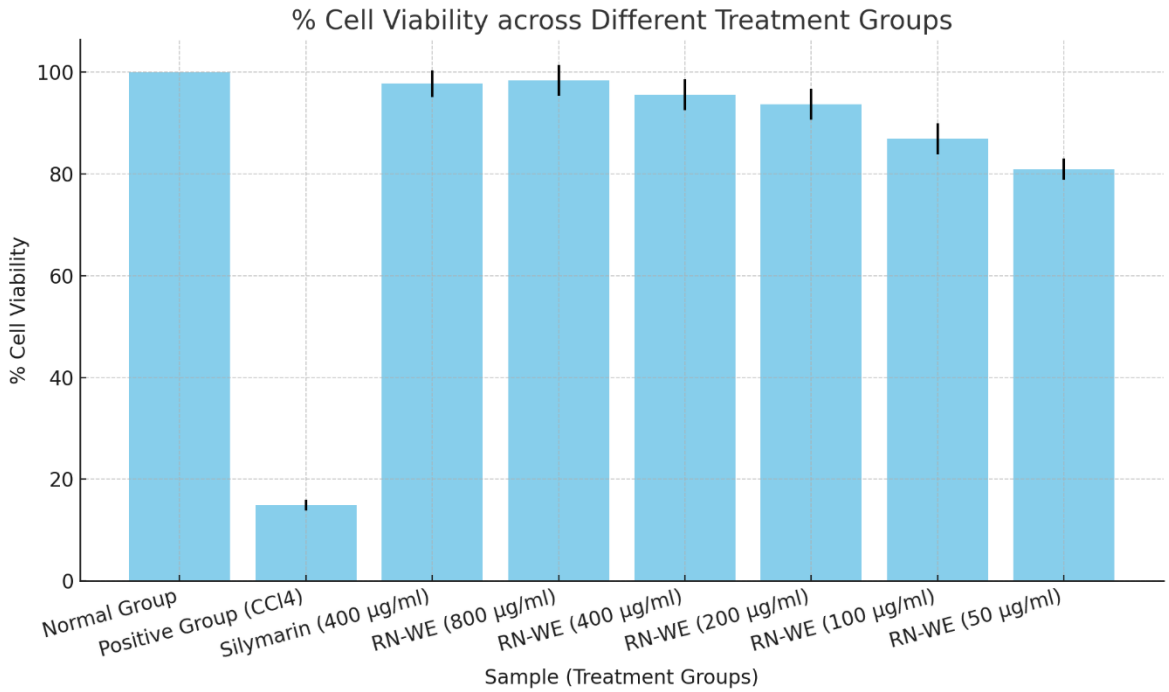

Figure 3. The hepatoprotective effect of RN-WE in CCl4 induced injury in HepG2 cells.

CONCLUSIONS

In conclusion, the comprehensive evaluation of *Rumex napalensis* leaves water extract (RN-WE) highlighted its significant antioxidant and hepatoprotective properties. The extraction process effectively preserved the integrity and potency of bioactive compounds, as evidenced by the high yield and diverse phytochemical profile of the extract. RN-WE exhibited potent antioxidant activity, surpassing standard antioxidants in certain assays, and demonstrated significant hepatoprotective effects against CCl4-induced hepatic injury. These findings provided valuable insights into the potential therapeutic applications of RN-WE in mitigating oxidative stress-related diseases and liver disorders. RN-WE presented a promising natural alternative for antioxidant therapy and liver protection, offering potential benefits for public health and pharmaceutical industries. Further research, including in vivo studies and clinical trials, is warranted to validate the efficacy, safety, and mechanisms of action of RN-WE for its integration into clinical practice and healthcare interventions.

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