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evaluation of cardioprotective activity of centella asiatica l on experimentally induced oxidative stress

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Abstract

Nowadays, lifestyle and food habits have resulted in many complaints, leading to an increasing interest in identifying natural phytochemical compounds from plant species that can be used as medicines without significant side effects. Herbal medicines are multicomponent mixtures of organic and inorganic compounds, and their chemical constituents possess a pharmacological and antioxidant potential that could have the ability to protect vital organs from several ailments with less or no toxic Effect. The Present study aimed to evaluate the pharmacological and antioxidant activity of the well-known medicinal plant Centella Asiatica, which was extracted traditionally. The natural antioxidants in the aqueous plant extracts ameliorate liver and heart damage by consuming ethanol. Hence, this study concluded that the aqueous Extract of Centella Asiatica profoundly protected the vital organs. These findings support the fact that the active principles of Centella Asiatica significantly contributed to the authenticity of the development of new drugs that could alleviate society from many diseases due to oxidative stress by alcohol consumption. It is essential to evaluate the pharmacological properties, and the experimentation would be necessary to standardize and elucidate the exact mechanism of action and toxicity studies of the bioactive compounds present in aqueous Extract to be formulated as protective drugs to treat the alcoholics without any side effects.

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INTRODUCTION

The consumption of alcoholic beverages is widespread. Ethyl alcohol, often known as ethanol, is made from fermented grains, fruit juice, and honey. Early Egyptian civilization, China circa 7000 BC, India circa 3000–2000 B.C., Babylon, Greece, and South America as early as 2700 BC all used fermented liquids and alcoholic drinks. The sixteenth century saw a lot of therapeutic use for alcohol. Disparities in alcohol-related issues between racial and cultural groups are concerning

for public health. Alcoholism has a negative social and economic impact on society as a whole as well as on individuals. Nowadays, the majority of human societies worldwide habitually use alcoholic beverages [1].However, misuse of it is a global public health issue. Alcohol misuse affects almost 20 million people in the U.S., and it results in the death of 100,000 people every year. One of the leading causes of death for young men in India nowadays is alcohol abuse. The average age of participants at the onset of alcohol intake has shown a decline. India's sizable population has made it the world's third-largest market for alcoholic drinks. In India, per capita alcohol consumption climbed by 106.7% between 1970 and 1996 despite the country having a high percentage of lifetime abstainers (89.6%). Alcohol consumption has increased as a result of shifting social norms, urbanization, increased availability, high-intensity mass marketing, loosened regulations governing international trade, and low awareness [2].

MATERIALS AND METHODS

PROCUREMENT OF PLANT MATERIAL

Centella asiatica were chosen and gathered for the current study in plantain fields in and around Seshachalam Forest in Tirupathi, Andhra Pradesh, India. Botanist Rtd. Dr. Madhava Chetty of the Department of Botany at S.V. University in Tirupathi identified the voucher specimen. After thoroughly washing the leaves of Centella asiatica two or three times under running water, they were shade-dried, pounded into a powder, and then placed in a sterile container. The subsequent analyses were conducted independently for every plant and combination of formulations.

Plant extraction:

A rotary shaker was used to extract 5 kg of dried leaves using 1 L of several solvents, including ethanol, methanol, acetone, and water, and the leaves were left for 24 hours. It was then filtered and centrifuged for 15 minutes at 5000 rpm. In a water bath, the solvent was evaporated until it was scorched, collecting the supernatant. For future research, the dehydrated powder was kept in three airtight, sterile containers at 4ºC [3].

Physico-chemical analysis of Centella Asiatica

Determination of Foreign matter

A thin layer of 100g of Centella asiatica leaves was spread out. Even the unaided eye could recognize the alien material. It underwent separation, weighing, and computation.

$$\%\ of\ Foreign\ organic\ matter = \frac{(M1 - M)}{M2} X\ 100$$

Determination of moisture content

100 g of plant material was precisely weighed in a tarred evaporating dish, dried for five hours at 105°C, and then weighed again. This was done without any prior drying. The process of drying and weighing was carried out hourly until the discrepancies between the two subsequent weight measurements nearly matched and did not exceed 0.25 percent. The substance was chilled in desiccators for 30 minutes after reaching a consistent weight. It was computed what the percentage of moisture content was [4].

Determination of Ash value

To calculate the ash value, three separate techniques can be used to measure the total ash, acid-insoluble ash, and water-soluble ash. The ash value is valid when evaluating the quality and purity of the raw plant material in powder form.

Qualitative analysis of phytochemicals

The plant extracts were subjected to a preliminary phytochemical screening using the procedures and evaluation standards provided by Harborne and Kokate[5].

In vitro Antioxidants Analysis

DPPH Radical Scavenging Assay

When DPPH and methanol or ethanol react, a purple-colored DPPH radical is produced. The sample's antioxidant decreases the hue, readable at 517 nm, when it reacts with the DPPH radical. One milliliter of the DPPH 0.004% methanol solution was combined with one milliliter of Centella asiatica aqueous extracts at different concentrations (10, 20, 30, 40, and 50 µg/ml). 50 µg/ml of ascorbic acid was utilized as the reference. After vortexing, the reactants were allowed to sit at room temperature for 30 minutes in the dark. Excluding samples, the control was constructed similarly. At 517 nm, the absorbance was determined using a spectrophotometer. The assay was run three times. A percentage of the

DPPH radical scavenging activity was determined using the following formula:

$$\%\ \text{of DPPH scavenged} = \frac{[\text{Abs control} - \text{Abs sample}]}{[\text{Abs control}]} X\ 100$$

The IC50 value was calculated: the inhibitory concentration at which the antioxidant activity is 50%.[6]

Reducing Power Assay

Potassium ferrocyanide (Fe2+) is produced when plant extract with reducing potential combines with potassium ferricyanide (Fe3+) to form ferric chloride, a ferric ferrous complex with an absorbance maximum of 700 nm.

$$\text{Potassium ferricynaide} + \text{Ferri chloride} \xrightarrow{\text{Antioxidant}} \text{Potassium ferricynaide} + \text{Ferrous chloride}$$

Centella Asiatica was combined with 2.5 ml of 0.2M phosphate buffer (pH 6.6) and 1% potassium ferricyanide at different concentrations (20, 40, 60, 80, and 100 µg/ml). This combination was maintained at 50ºC in a water bath for twenty minutes. After cooling, 2.5 ml of 10% trichloroacetic acid was added, and the mixture was centrifuged for 10 minutes at 3000 rpm. A freshly made 0.1% ferric chloride solution (0.5 ml) and 2.5 ml of distilled water were combined with the upper layer of the solution. At 700 nm, the absorbance was measured. Except for the samples, the control was prepared similarly. The standard was ascorbic acid. The assay was run three times. Increasing the reaction mixture's absorbance signifies a more substantial reducing power.

Hydrogen Peroxide Scavenging Activity

The four-milliliter reaction mixture was composed of 0.6 milliliters of 40 mM hydrogen peroxide and 0.2–1.0 milliliter of Centella asiatica aqueous extracts (20µg/ml to 100µg/ml), which were diluted to 3.4 milliliters using phosphate buffer. At 230 nm, the absorbance was measured. The standard was ascorbic acid (10 mg/100 ml), and the blank solution was phosphate buffer (pH 7.4).

The following formula was used to determine the percentage of hydrogen peroxide scavenging activity:

$$\%\ of\ Scavenging\ activity = \frac{[Abs\ control - Abs\ of\ test]}{[Abs\ control]} X\ 100$$

Superoxide Radical Scavenging Activity

The phenazine methosulfate: nicotinamides adenine dinucleotide system was employed to produce superoxide anion. Superoxide anions were produced using the NADH system. Nitroblue tetrazolium was then reduced by the superoxide anions, creating a chromogenic product detected at 560 nm. At a total volume of 3 ml, Centella Asiatica was combined at different doses (20, 40, 60, 80, and 100 µg/ml) with 1 ml of nitroblue tetrazolium (156 µm), 1 ml of NADH (468 µm), and 67 mM, pH 7.8 phosphate buffer. Analogously, ascorbic acid (50 µg/ml) was the standard. For five minutes, the reaction mixture was incubated at 25°C. Control was prepared similarly, excluding samples. The percentage of inhibition was determined by comparing the control and test sample results.

$$\%\ of\ superoxide\ radical\ Scavenging\ activity = \frac{[Abs\ control - Abs\ of\ test]}{[Abs\ control]} X\ 100$$

Nitric Oxide Scavenging Activity

Nitric acid is transformed into nitrous acid and then into nitrite ions (NO-), which diazotize when combined with sulphuric acid and naphthyl ethylene diamine dichloride to produce a pink hue that is detectable at 546 nm. Centella asiatica aqueous extract was mixed with 2 milliliters of sodium nitroprusside (10 mM in 0.5 milliliters of phosphate-buffered saline at pH 7.4) at different doses (20, 40, 60, 80, and 100 µg/ml). The standard was 50 µg/ml of ascorbic acid.

For 2.30 hours, the mixture was incubated at 25°C. 0.5 milliliter of the reactants were removed after incubation, and 1 milliliter of sulphanilic acid reagent (0.33% in 20% glacial acetic acid) was combined and left to stand at room temperature for five minutes to allow the diazotization reaction to be fully completed. 1 ml of naphthyl ethylene diamine dichloride (0.1%w/v) was added, and the mixture was allowed to stand for 30 minutes at room temperature. Control was prepared similarly, excluding samples. Absorbance was measured at 540 nm in a spectrophotometer. The assay was carried out in triplicates. The nitric

oxide inhibition percentage was calculated using the formula

$$Nitric\ oxide\ inhibition\ \% = \frac{[Abs\ control - Abs\ of\ sample]}{[Abs\ control]} X\ 100$$

The IC50 value was calculated: the inhibitory concentration at which the antioxidant activity is 50%.

Biochemical Analysis and *In vivo* Antioxidant Assays

Preparation of sample for in-vivo antioxidant assays

Homogenising the liver (100 mg/ml) in 50 mM phosphate buffer (pH: 7.0) was done. After centrifuging the homogenate for ten minutes at 10,000 rpm, the supernatant was collected and used for the subsequent analysis [8].

Estimation of lipid peroxide

By precipitating serum protein with 0.02% TCA, lipids were separated. Malondialdehyde is produced by interacting with TBA in an acetic acid solution to determine the degree of lipid peroxidation. Absorption at 532 nm was used to measure the reaction product. 1.5 ml of 20% acetic acid, 0.2 ml of sodium dodecyl sulfate, and 1.5 ml of TBA were combined with 0.2 ml of tissue homogenate. 4 ml of water was added to the capacity. For one hour, the tubes were incubated in a water bath. After the tubes had cooled, 5 milliliters of the butanol pyridine reagent were added. For ten minutes, it was centrifuged at 3000 rpm. Two layers were distinguished. At 530 nm, the phase of color extraction (pink) was read. Micromoles of malondialdehyde formed/gm of Tissue was the unit of measurement for the lipid peroxide level. (20 percent acetic acid; 0.67 percent thiobarbituric acid); 10% sodium dodecyl sulphate; 15:1 butanol-pyridine reagent; tissue homogenate) [9].

Assay of superoxide dismutase

As a first line of defense against harm from free radicals, SOD scavenges the superoxide radical. The method of Mishra & Frigivich (1972) was utilized to measure SOD in the liver homogenate. This method was based on an enzyme's oxidation of epinephrine adrenochrome transition. The dismutation of superoxide anions (O2-) into hydrogen peroxide and oxygen molecules is catalyzed by SOD in the following ways.

$$2H_2O + 2O_2 - \ \rightarrow 2H_2O_2 + O_2$$

When SOD activity is present, the superoxide anion (O2) combines with peroxide to generate hydroxyl radical, which is harmful. 0.75 ml of ethanol, 0.15 ml of ice-cold chloroform, and 0.1 ml of tissue homogenate were combined. After thoroughly mixing the reaction mixture, it was centrifuged for ten minutes at 3000 rpm. 0.5 ml of EDTA solution and 1 ml of buffer were added to 0.5 ml of supernatant. 0.5 milliliters of epinephrine were added to start the reaction, and the absorbance increased to 480 nm. (PH 10.2; 0.1M carbonate-bicarbonate buffer; 0.6M EDTA solution; 1.8M freshly produced epinephrine; absolute ethanol; Chloroform; Tissue homogenate) [10].

Assay of catalase

Hydrogen peroxide's U.V. absorption may be measured at 240 nm, and when catalase breaks it down, its absorbance drops. It is possible to calculate the enzyme activity from the decrease in absorbance. To start the enzyme reaction, 0.05 ml of tissue homogenate was added to 1.2 ml phosphate buffer and 1.0 ml hydrogen peroxide. At 620 nm, the absorbance drop was monitored for three minutes at intervals of 30 seconds. The enzyme blank and 1.0 milliliter of deionized water were run concurrently. The catalase activity was reported as nm of H2O2 used \min\mg\protein. (0.05g of potassium dichromate, an acetic acid reagent, was dissolved in 1 ml of distilled water.) Hydrogen peroxide was dissolved in a 0.2 M solution in phosphate buffer (0.01 M). After adding 3 milliliters of acetic acid, distilled water was used to get the solution up to 15 milliliters.)

Estimation of glutathione reductase

Glutathione (GSSG) to reduced glutathione (GSH) conservation requires a certain amount of NADPH, which determines the G.R. activity. The enzyme glutathione reductase catalyzes the reaction. The reaction mixture was increased to 3 ml with distilled water and contained 1 ml of phosphate buffer, 0.5 ml of EDTA, 0.5 ml of GSSG, and 0.2 ml of NADPH. The change in optical density at 340 nm was tracked for two minutes at 30-second intervals after adding 0.1 ml of tissue homogenate.

The expression for one unit of the enzyme activity was moles of NADPH oxidized / min/mg protein. (Tissue homogenate, GSSG (0.012 M), NADPH (0.003 M), EDTA (0.25 M), phosphate buffer (0.3 M; pH 6.5), and glutathione oxidized [11].

Assay of reduced glutathione

With dithionitrobenzene (DTNB), the acid-soluble sulfhydryl group—a non-protein thiol of which over 93% is reduced glutathione—forms a compound with a yellow color. At 412 nm, the colored complex's absorbance was measured. 0.5 ml of 20% TCA was added to 0.5 ml of homogenate. The mixture was thoroughly mixed to ensure that all of the protein precipitated, and it was centrifuged for 10 minutes at 3000 rpm. To make a final volume of 4.0 ml with distilled water, 2.0 ml of DTNB reagent and 0.2 ml of phosphate buffer were added to 0.5 ml of supernatant. A series of standards were treated similarly to find the glutathione content, and the absorbance was measured at 412 nm against a blank containing TCA. To express the amount of glutathione, it was calculated as follows: 10 mg of reduced glutathione dissolved in 100 milliliters of water (standard solution); 10% TCA; 0.6 mM 5,5' Di this-2-nitrobenzoic acid (DTNB) in 0.2 M sodium phosphate; 0.2 M phosphate; buffer pH 8.0 [12].

The activity of glutathione peroxidase

An enzyme preparation was allowed to react with H2O2 in the presence of GSH for a predetermined duration. At 420 nm, the GSH content that remained after the reaction was read immediately. The reaction mixture was incubated at 37°C for 10 minutes. It contained 0.2 ml of 0.8 mM EDTA, 0.1 ml of 10 mM sodium azide, 0.1 ml of 2.5 mM H2O2, 0.2 ml of GSH, 0.4 ml of 0.4 mM phosphate buffer (pH 7.0), and 0.2 ml of homogenate. After adding 0.5 ml of 10% TCA, the reaction was stopped, and the tubes were centrifuged at 2000 rpm. The GPx activity was measured in µmoles of oxidized glutathione per minute per milligram of protein. (Tissue homogenate; 0.3M sodium hydrogen phosphate; 1.0 ml of DTNB; 0.4 M EDTA; 10mM sodium azide; 2mM reduced glutathione; 1mM H2O2; 10%TCA) [13].

(HDL) cholesterol

The phosphotungstic acid (PTA) precipitate was used to assess HDL cholesterol. Phosphotungstic acid precipitated the serum's low-density lipoprotein (LDL) and very low-density lipoprotein (VLDL) fractions. The HDL in the supernatant was then separated by centrifugation, and its cholesterol concentration was determined. The enzyme cholesterol ester hydrolase (CEH) hydrolyzed the cholesterol esters. The oxidation of cholesterol produced cholest-4-en-3-one and hydrogen peroxide. When peroxidase is present, hydrogen peroxide combines with phenol and 4-amino antipyrine to form a red complex, which can be measured at 505 nm using a spectrophotometer. The absorbance correlated with the content of HDL cholesterol.

Estimation of lactate dehydrogenase

LDH activity in serum is proportional to the rise in absorbance caused by the reduction of NAD. LDH catalyzes the conversion of lactate to pyruvate and concurrently reduces NAD to NADH.

$$\text{Lactate} + \text{NAD} \qquad \text{Pyruvate} + \text{NADH} + \text{H}^{+}$$

Ten ml of working reagent was mixed with 0.05 ml of serum. The first absorbance was measured precisely at one minute, then at 30,60, and 90 seconds at 340nm. Change in mean absorbance per minute was calculated.

$$\text{LDH activity} = \text{Change in absorbance /minute X F}$$

(Reagent 1: 5mmol/L and 50mmol/L of lactic acid; Reagent 2: buffered substrate mixed two reagents gently) [14].

Histopathological examination

Hematoxylin (Haris's) crystals: 1g, Alcohol 95%: 10ml, Ammonium alum or potash: 20g, Distilled water: 200ml, Mercuric oxide: 0.5g). Eosin is used as a counter stain that stains cytoplasm rose-red colored. (Eosin (water soluble): 1g, Distilled water: 80 ml, 95% alcohol: 320ml, Glacial acetic acid: 0.4ml). The dehydrated tissues were stained using hematoxylin and eosin dyes. The dried slide was dipped into a hematoxylin stain solution twice and rinsed with distilled water. Then, it was dipped in acid alcohol reagent and drained, followed by eosin dye, and drained in isopropyl alcohol and xylene. After the complete draining of xylene, the coverslip was placed without air bubbles and was viewed under the light microscope (10X)[15].

RESULTS AND DISCUSSION

Analysis of plant material

Table 1 Physicochemical analysis of Centella Asiatica

S. No	Parameters	Value (%) W/W of Centella asiatica
1.	Foreign matter	2.78 ± 0.21
2	Moisture content	7.81 ± 0.92
3	Total ash	5.27 ± 0.21
4	Water soluble ash	2.8 ± 0.12
5	Acid insoluble ash	1.72 ± 0.15

Values are means ± SD; n = 3; The physicochemical analysis of leaves of Centella Asiatica powder was tabulated (**Table 1**). In the present study, foreign matter (2.78 %) and moisture content (7.81%) in the crude plant powder were higher in Centella asiatica.Centella asiatica has a slightly higher value of total ash (5.27 %), water-soluble ash (2.8 %), and acid-insoluble ash (1.72 %)The plant's inorganic materials were total ash, water-soluble ash, and acid-insoluble ash. Increased ash content indicated the considerable presence of inorganic materials in Centella asiatica.

In vitro antioxidant assay

DPPH Assay of Centella asiatica

Table 3 and **Figure 1** demonstrate how Centella Asiatica scavenged the DPPH free radical. At a low concentration of 10µg/ml, Centella Asiatica exhibited 25% of its activity, whereas at a high concentration of 50µg/ml, it displayed 60%. The IC50 value was determined to be 30µg/ml.

Table 3 DPPH Assay of Centella asiatica

S. No	Concentration of plant Extract (µg/ml)	% of DPPH scavenging Centella asiatica
1.	10	25 ± 1.20
2.	20	42.24 ± 0.98
3.	30	52.24 ± 1.25
4.	40	58.42 ± 0.99
5.	50	62.45 ± 1.25
6	Ascorib Acid (50 µg/ml)	65.27 ± 1.26

Values are means ± SD; n = 3

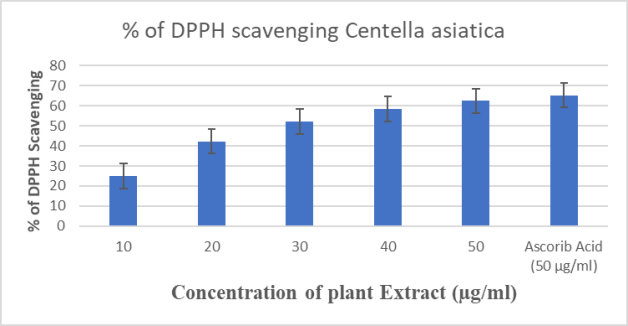


Figure 1 DPPH Assay of Centella asiatica

The outcomes were similar to those obtained with 65.27µg/ml of conventional ascorbic acid. The plant extracts' ability to donate either hydrogen or electrons was the cause of the decrease in DPPH.

Preliminary phytochemical screening of Centella asiatica

Table 2 Centella asiatica preliminary phytochemical screening

S.No	Name of the Test	Ethanol	Methanol	Acetone	Water
1	Alkaloids	+	-	++	-
2	Flavonoid	-	-	-	+
3	Steroids – Liberman Buc hard's test	+	+	-	+
4	Cardiac glycosides	+	+	++	+
5	Terpenoids – Salkowski test	++	+	+	+
6	Tannin	+	+	++	++
7	Saponin	-	-	-	+
8	Lignin	-	-	-	-
9	Anthraquinones	++	-	++	+
10	Protein	+	+	+	+
11	Carbohydrates	++	++	++	++
12	Reducing sugars	+	++	+	+++
		9	7	8	11

+ indicates Presence & - Indicates absence

Centella asiatica aqueous Extract's antioxidant capacity was assessed using a variety of in-vitro investigations. DPPH, a free radical, must take on an electron or a hydrogen radical to create stable molecules.

Reducing power assay of Centella asiatica

Table 4 Centella asiatica reducing power assay

S. No	Concentration of plant Extract (µg/ml)	Reducing power assay of Centella asiatica
1.	20	26.12±.24
2.	40	45.21±0.29
3.	60	81.25±0.89
4.	80	89.24±1.02
5.	100	92.14±0.97
6	Ascorib Acid (50 µg/ml)	82.45±1.24

Values are means ± SD; n = 3;

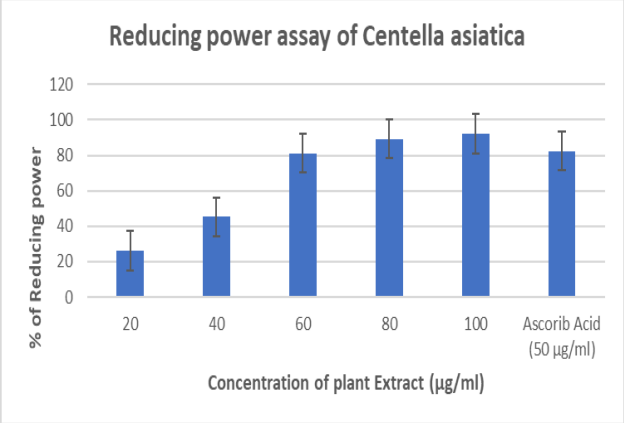


Figure 2 Centella asiatica reducing power assay

Table 4 and **Figure 2** show the results of the Centella asiatica extract's reducing power assay. At low concentrations of 26.12 µg/ml, Centella Asiatica demonstrated 24% reducing power; at high concentrations of 100 µg/ml, it demonstrated 92.14%. The results showed that the IC50 value for both extracts was 45µg/ml. An essential mechanism of antioxidant action is electron-donating activity, commonly indicated by the reducing power assay. In the reducing power test, the Extract's constituent can donate an electron to lower the Fe3+ to Fe2+.

At 700 nm, the generation of Fe3+ was quantified. With ascorbic acid serving as a reference, researchers examined the reductive capacities of the Mimusopselengi leaf extract. Higher absorbance implies higher reducing ability. It was discovered that the Mimusopselengi extract has a significant, dose-dependent reduction power. The current investigation's findings coincide with those of previous ones.

Hydrogen peroxide assay of Centella asiatica

Table 5 Hydrogen peroxide assay of Centella asiatica

S. No	Concentration of plant Extract (µg/ml)	Hydrogen Peroxide scavenging assay
1.	20	11.28±.0.89
2.	40	26.8±1.25
3.	60	55.69±0.45
4.	80	71.26±0.85
5.	100	82.15±1.25
6	Ascorib Acid (50 µg/ml)	85.25±1.26

Values are means ± SD; n = 3;

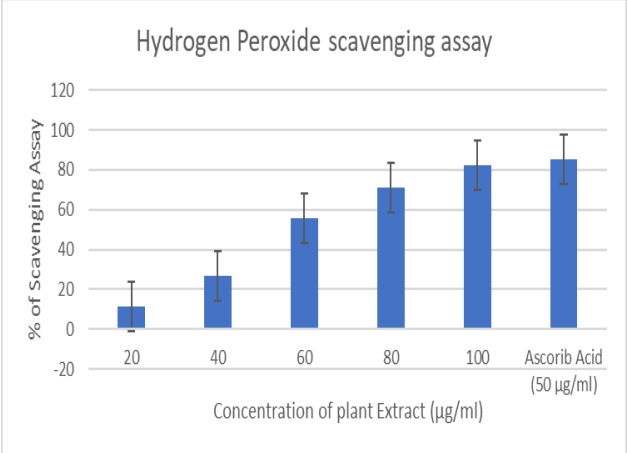


Figure 3 Hydrogen peroxide assay of Centella asiatica

Table 5 and **Figure 3** show Centella asiatica's capacity to scavenge H2O2. The Extract exhibited moderate action at low concentrations (20µg/ml) and maximum activity at high concentrations (100µg/ml). At a low concentration of 20µg/ml, Centella asiatica demonstrated 11.28% H2O2 scavenging activity. At a high concentration of 100µg/ml, Centella asiatica displayed 82.15%. Centella asiatica was discovered to have an IC50 value of 46.9µg/ml. Even though hydrogen peroxide is a weak oxidizing agent, its capacity to cross biological membranes makes it significant. Once inside the cell, it can likely combine with Fe2+ and Cu2+ ions to create hydroxyl radicals, which may be the source of many of its harmful effects.

Superoxide radical scavenging potential of Centella asiatica

Table 6 Centella asiatica's potential to scavenge superoxide radicals

S. No	Concentration of plant Extract (µg/ml)	Hydrogen Peroxide scavenging assay
1.	20	22.25±1.10
2.	40	24.58±1.99
3.	60	32.72±2.07
4.	80	46.67±1.22
5.	100	53.65±1.51
6	Ascorib Acid (50 µg/ml)	80.02±147

Values are means ± SD; n = 3;

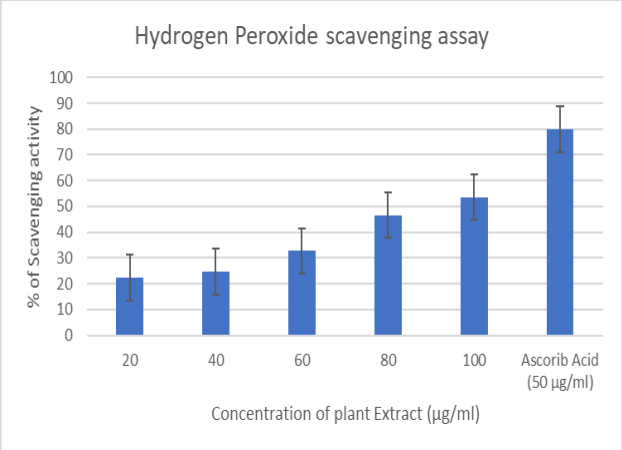


Figure 4 Centella asiatica's potential to scavenge superoxide radicals

It was observed from **Table 6** and **Figure 4** that the Centella asiatica aqueous extracts may inhibit the superoxide radicals.

Centella asiatica exhibited 53.65 percent activity at the high concentration (100µg/ml), similar to the standard. Centella asiatica was discovered to have an IC50 value of 92µg/ml. The superoxide anion radical is one of the most potent reactive oxygen species among the free radicals produced. Despite being the leading free radical in the biological system, oxygen is not very reactive. Nevertheless, the system transforms it into more reactive species, such as O.H. and H2O2 radicals, which harm biomolecules and cause chronic illnesses. Superoxide anion is involved in generating additional free radicals that damage cells and play a significant function in plant tissues. The plant extract under investigation was successful in scavenging the superoxide radical.

Potential for inhibiting nitric oxide in Centella asiatica extract

Table 7 Potential for inhibiting nitric oxide in Centella asiatica extract

S. No	Concentration of plant Extract (µg/ml)	Nitric oxide inhibition potential of Centella asiatica
1.	20	9.21±1.02
2.	40	18.23±1.26
3.	60	22.72±2.07
4.	80	42.02±1.22
5.	100	53.65±0.26
6	Ascorib Acid (50 µg/ml)	70.02±1.05

Values are means ± SD; n = 3;

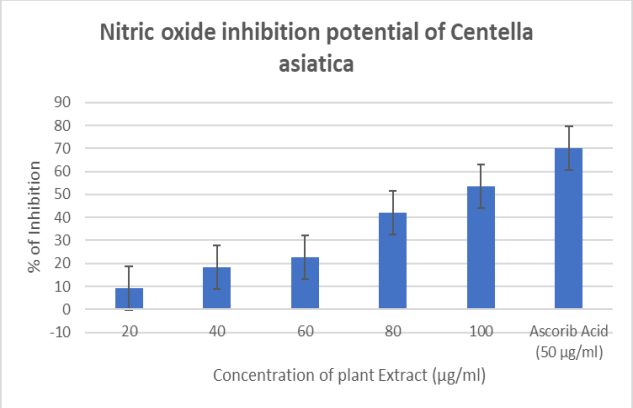


Figure 5 Potential for inhibiting nitric oxide in Centella asiatica extract

Figure 5 and **Table 7** displayed Centella asiatica's NO inhibition potentials. Centella asiatica (20µg/ml) demonstrated 9.21% activity at low concentrations. Both plant extracts demonstrated 53.65% of their potential to suppress NO at high concentrations (100µg/ml). The value of IC50 was determined to be 92µg/ml. Reactive peroxynitrile (ONOO -), produced when NO and O2 mix, increases the toxicity and damage that are already induced and can result in significant toxic interactions with biomolecules. Macrophages and endothelial cells generate the free radical known as NO. When aerobic conditions are met, nitric oxide is a volatile species. It combines with oxygen to form the stable products nitrates and nitrites using NO2, N2O4, and N3O4 as intermediates. The Griess reagent is used to estimate it. The amount of nitrous acid will drop when the scavenger test chemical is present. In a biological system, free radicals are continuously created. These radicals can seriously harm biomolecules and tissues,

leading to various disorders, including degenerative diseases and widespread lysis. The plant had a moderate ability to scavenge free radicals in NO. The test samples' concentration was raised to enhance the NO free radical scavenging activity.

IN VIVO ANTIOXIDANT AND BIOCHEMICAL ANALYSIS

Effect of aqueous Extract of Centella asiatica on body weight in control and ethanol-administered rats

asiatica (750 mg/kg b.w), the body weight increased to 4.9 %. Compared with the treated Group, In patients with venous hypertension, centella asiatica extract improved capillary permeability and the microcirculatory impact. The Extract was demonstrated to impact lipolytic activity, which raised the amount of cyclic adenosine monophosphate (cAMP) in human adipocytes and may help with the slimming Effect. One of the slimming liposomes' potent microcirculation activators is Centella asiatica extract.

Table 8 Effect of aqueous Extract of Centella asiatica on body weight in control and ethanol-administered rats

Groups	Treatment	Body Weight in grams		
		Initial	Final	% of Change
I	Normal	126.23 ± 2.12	138.24 ±1.58	9.5
II	Ethanol (3 g/kg BWP.o)	135.21 ±1.98	126.21±2.31	-7.1
III	Silymarin (250mg/ kg/bwp.o)	128.62 ±2.14	132.21 ± 1.25	5.9
IV	Centella asiatica (750 mg /kg bw p.o)	138.23 ±1.85	144.65 ± 2.56	4.9

Values are means ± SD; n = 6; Group II compared with Group I; Group III & IV –compared with Group II; ANOVA statistically significantly different at P < 0.05

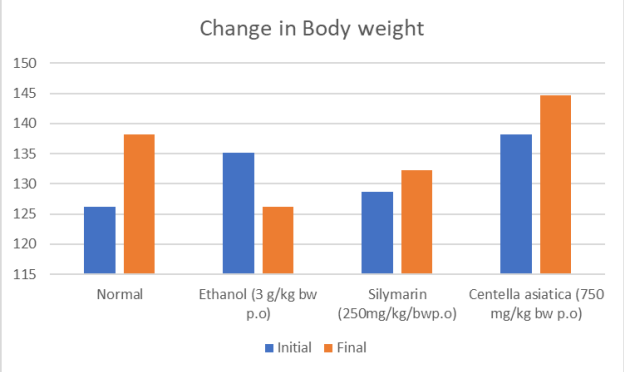


Figure 6 Effect of aqueous Extract of Centella asiatica on body weight in control and ethanol-administered rats

Table 8 and **Figure 6** illustrate the body weight of control, ethanol-induced, standard drug (Silymarin 250mg/kg bw), Centella asiatica (750 mg/kg b.wt treated rats. The body weight of rats decreased by – 7.1 % in the ethanol-induced Group (Group II - Negative control group) compared to Group I (Positive control group). On administration of aqueous Extract of Centella

Effect of aqueous Extract of Centella asiatica on liver weight in control and ethanol-administered rats

Table 9 Effect of aqueous Extract of Centella asiatica on liver weight in control and ethanol-administered rats

Groups	Treatment	Liver Weight in grams	
		Final	% of Change
I	Normal	3.25 ±0.58	-
II	Ethanol (3 g/kg BWP.o)	1.98 ±0.21	-39.07
III	Silymarin (250mg/kg/bwp.o)	3.10. ± 0.15	56.5
IV	Centella asiatica (750 mg/kg bwp.o)	2.65 ± 0.56	33.83

Values are means ± SD; n = 6; Group II compared with Group I; Group III &IV, compared with Group II; ANOVA statistically significantly different at P < 0.05

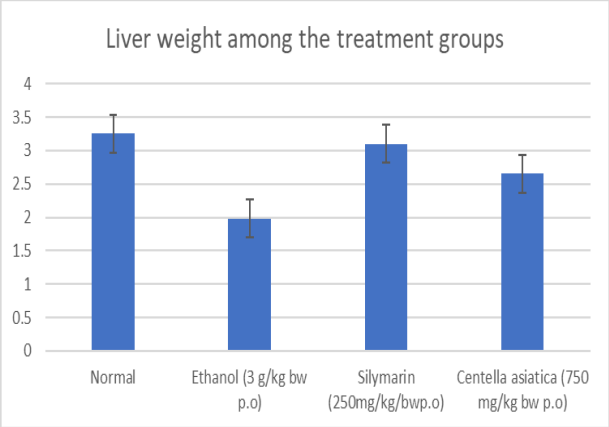


Figure 7 Effect of aqueous Extract of Centella asiatica on liver weight in control and ethanol-administered rats

Table 9 and **Figure 7** showed the liver weight of control, ethanol-induced, standard drug, Centella asiatica (750 mg/kg b.w), treated rats. The liver weight of the ethanol-induced Group (Group II - Negative control group) decreased by 39.07 % compared to that of Group I (Positive control group) rats. On administration of an aqueous Extract of Centella asiatica (750 mg/kg b.w), the liver weight of rats was increased to 33.83 %, respectively. The standard drug Silymarin treated Group showed an increase in liver weight by 33.83 %. Alcohol intoxication increases lipid peroxide production (LPO) in various tissues, and it is an indication of oxidative stress in the liver.

Effect of aqueous Extract of Centella asiatica on TBARS, SOD, CAT, GSH, GPx, and G.R. in control and ethanol-administered rats

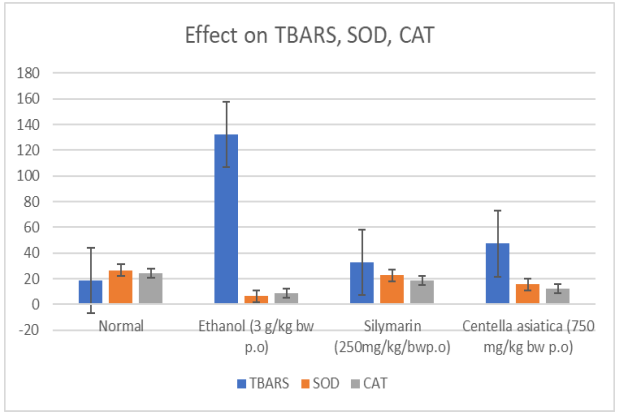


Figure 8 Effect of aqueous Extract of Centella asiatica on TBARS, SOD, CAT

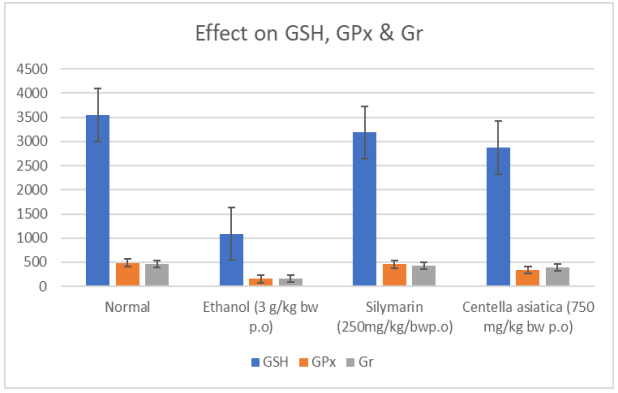


Figure 9 Effect of aqueous Extract of Centella asiatica on GSH, GPx & Gr

Table 10 and **Figure 8** and **Figure 9** demonstrated the levels of TBARS, SOD, CAT, GSH,

Table 10 Effect of aqueous Extract of Centella asiatica on TBARS, SOD, CAT, GSH, GPx and G.R. in control and ethanol-administered rats

Groups	Treatment	Parameters					
		TBARS	SOD	CAT	GSH	GPx	Gr
I	Normal	18.5±2.24	26.54±2.45	24.56±2.58	3546±102	487±2.1	460±12.1
II	Ethanol (3 g/kg BWP.o)	132.02±4.98	6.35±1.25	8.65±2.01	1089±98.1	154±6.2	160±4.5
III	Silymarin (250mg/kg/ bwp.o)	32.54±2.58	22.54±3.64	18.56±2.5	3187±102	457±8.2	427±12.1
IV	Centella asiatica (750 mg/kg bwp.o)	47.25±3.56	15.45±4.21	12.54±1.58	2879±98.4	341±9.4	387±10.2

Values are means ± SD; n = 6; Group II compared with Group I; Group III & IV, compared with Group II; ANOVA statistically significantly different at P < 0.05

GPx, and G.R. of control, ethanol-induced, standard drug (Silymarin 250mg/kg bw), Centella Asiatica (750 mg/kg b.w)

The level of TBARS indicates the stage of liver introduction, which significantly increased in Group II animals compared to expected.

The level of TBARS in Group I was 18.5±2.24 µM of MDA/g of Tissue (Positive control), and in the induced Group was 132.02±4.98µM of MDA/g of Tissue (Group II - Negative control). On administration of aqueous Extract of Centella asiatica (750 mg/kg b.w), the level of TBARS was 47.25±3.56µM of MDA/g of Tissue respectively, which was comparable with Silymarin treated Group where the level of TBARS was 32.54±2.58µM of MDA/g of Tissue. Among the treated groups, the mixed formulation showed a significant decrease in TBARS.

The level of Thio Barbituric Acid Reactive Substance (TBARS) indicated the intoxication stage of the liver. It was significantly increased in group II animals when compared to expected. Other treated groups showed a marked reduction in the level of TBARS.The enzymatic antioxidant SOD activity in Group I was 26.54±2.45µM of Epinephrine/g of Tissue (Positive control), and in the ethanol-induced Group was 6.35±1.25µM of Epinephrine /g of Tissue (Group II - Negative control). On administration of aqueous Extract of Centella asiatica (750 mg/kg b.w), the activity of SOD was 15.45±4.21µM of Epinephrine/g of Tissue, respectively, which was comparable with Silymarin treated Group where the activity was 22.54±3.64µM Epinephrine/g of Tissue. Among the treated groups, the mixed formulation showed a significant increase in the activity of SOD.

The activity of CAT in Group I was 24.56±2.58 µM H2O2 hydrolyzed/minute/g of Tissue (Positive control), and in the induced Group was 8.65±2.01 µM H2O2 hydrolyzed/minute/g of Tissue (Group II - Negative control). On administration of aqueous Extract of Centella asiatica (750 mg/kg b.w), the activity of CAT was 12.54±1.58µM H2O2 hydrolyzed/minute/g of Tissue which was comparable with Silymarin treated Group where the activity was 18.56±2.5µM H2O2 hydrolyzed/minute/g of Tissue. Among the treated groups, the mixed formulation showed a significant increase in the activity of CAT.SOD activity in hepatic tissues of ethanol-treated Wistar rats had four-fold decreased activity when compared to the standard Group, whereas different doses of aqueous plant extract treatment showed increased activity of SOD

Enzymes, including glutathione peroxidase (GPx), catalase (CAT), and superoxide dismutase (SOD), are found in plasma and the liver and contribute to the antioxidant defense system.

The level of GSH in Group I was 3546±102µg /g of Tissue (Positive control), and in the ethanol-induced Group was 1089±98.1 µg /g of Tissue µ (Group II - Negative control). On administration of an aqueous Extract of Centella asiatica (750 mg/kg b.w), the level of GSH was 2879±98.4 µg /g of Tissue, which was comparable with Silymarin treated Group where the level of GSH was 3187±102µg /g of Tissue. Among the treated groups, the mixed formulation showed a significant increase in the level of GSH.

GSH is a major non-protein thiol in living organisms that plays a vital role in coordinating the innate antioxidant defense process in the body system and is involved in redox and detoxification reactions to maintain the structure and functions of normal cells. Excessive lipid peroxidation causes increased consumption. GSH functions as a potent nucleophile and antioxidant, essential for cellular defence mechanisms such as detoxifying reactive oxygen species (ROS), conjugating and excreting harmful compounds, and managing the cascade of inflammatory cytokines. Alcohol-treated rats had considerably lower GSH levels, indicating that alcohol consumption lowers GSH concentrations.The level of GPx in Group I was 487±2.1g of GSH oxidized /minute /g of Tissue (Positive control), and in the ethanol-induced Group was 154±6.2g of GSH oxidized /minute /g of Tissue (Group II - Negative control). On administration of aqueous Extract of Centella asiatica (750 mg/kg b.w), the level of GPx was 341±9.4 of GSH oxidized /minute /g of Tissue, which was comparable with Silymarin treated Group where the level of GPx was 457±8.2g of GSH oxidized /minute /minute/g of Tissue. Among the treated groups, the mixed formulation showed a significant increase in the level of GPx.The level of G.R. in Group I was 460±12.1µg of GSH produced / minute / g of Tissue (Positive control), and in the induced Group was 160±4.5µg of GSH produced / minute / g of Tissue (Group II - Negative control). On administration of aqueous Extract of Centella asiatica (750 mg/kg b.w) and Phyllanthus amarus

(100, 200, 300 mg/kg b.w) level of G.R. 387±10.2.1 µg of GSH produced / minute / g of Tissue which was comparable with Silymarin treated Group where the level of G.R. was 427±12.1µg of GSH produced / minute / g of Tissue. Among the treated groups, the mixed formulation showed a significant increase in the level of G.R.The production of free radicals that led to the peroxidation of membrane lipids may have contributed to the rise in TBARS levels in the liver. Ethanol's antioxidant qualities caused changes in the levels of GSH, catalase, SOD, and TBARS. These characteristics may result from the presence of flavonoids, which can scavenge free radicals and shield the cell membrane from damage, as well as from the plant extracts' flavonoids, phenols, anthraquinones, tannins, alkaloids, and saponins. Because of their interactions with substances such as sex hormones, cortisones, diuretic steroids, vitamin D, and cardiac glycosides, saponins have a significant role in medicine. It is well-recognized that dietary polyphenols guard against degenerative illnesses and oxidative stress. Aqueous extracts of both plants showed the presence of secondary metabolites in qualitative and quantitative analysis in the present investigation, and according to other research findings, these are the compounds that act against the oxidative stress of free radicals due to the consumption of alcohol. The results of the present findings correlated with the above research findings.

Effect of aqueous Extract of Centella asiatica

The level of bilirubin (Total, direct, and indirect) was given in **Table 11** and shown in **Figure 10**. The bilirubins (Total, direct and indirect) in Group I (Positive control) were 0.4±0.10, 0.3±0.12, and 0.1±0.09 mg/dl, respectively, and in the ethanol-induced Group (Group II - Negative control) was 0.9±0.25, 0.3±0.12, 0.4±0.48mg/dl respectively.On administration of aqueous Extract of Centella asiatica (750 mg/kg b.w), the bilirubin content was (Total, direct, and indirect) 0.6±0.15, 0.3±0.24, 0.2±0.08. It was comparable with the treated Group, where the bilirubin level was 0.5±0.64, 0.3±0.17, and 0.1±0.07mg/dl. Among the treated groups.A statistically significant increase in total bilirubin in the acetaminophen-intoxicated Group's (Group II) serum compared to the control group (Group I). Total bilirubin levels were lowered by the Phyllanthus longiflorus ethanolic Extract at 200 mg/kg and 400 mg/kg. One of the most sensitive tests used is serum bilirubin when diagnosing hepatic disorders. It offers helpful details regarding the liver's health. The health and functioning of the hepatic cells are connected to the aberrant bilirubin level.

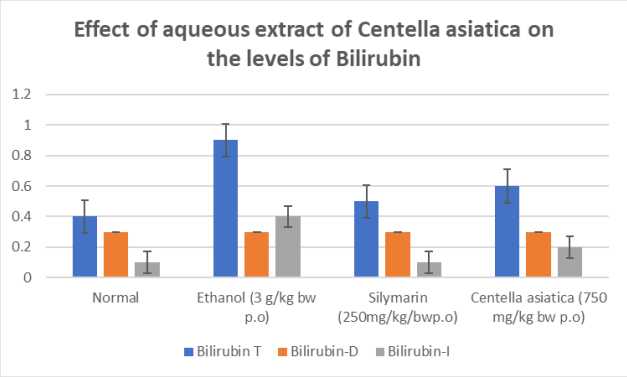


Figure 10 Effect of aqueous Extract of Centella asiatica on the levels of Bilirubins in Wistar rats

Table 12 and **Figure 11** represent the Liver marker enzymes (SGOT and SGPT) of control, ethanol-induced, standard drug (Silymarin 250mg/kg bw), Centella Asiatica (750 mg/kg b.w), Activity of SGOT and SGPT in Group I was 175±2.12, 65±2.45 U/L (Positive control) and in ethanol-induced group was 230±7.25, 90.21±3.14. (Group II - ve control). On administration of aqueous Extract of

Table 11 Effect of aqueous Extract of Centella asiatica on the levels of Bilirubins in Wistar rats

Groups	Treatment	Parameters		
		Bilirubin T	Bilirubin D	Bilirubin I
I	Normal	0.4±0.10	0.3±0.12	0.1±0.09
II	Ethanol (3 g/kg BWP.o)	0.9±0.25	0.3±0.12	0.4±0.48
III	Silymarin (250mg/kg/bwp.o)	0.5±0.64	0.3±0.17	0.1±0.07
IV	Centella asiatica (750 mg/kg bwp.o)	0.6±0.15	0.3±0.24	0.2±0.08

Values are means ± S.D.; n = 6; Group II compared with Group I; Group III & IV compared with Group II; ANOVA statistically significantly different at $P < 0.05$

Centella asiatica (750 mg/kg b.w), the SGPT of Centella asiatica was 182±3.12, 73.14±2.54 which was comparable with SGOT and SGPT of Silymarin treated group 168±3.85 and 63.36±2.14 U/L. Key cellular enzymes like AST, ALT, and ALP present in the liver cells and leak into the blood after liver injury were administered at higher amounts when ethanol was administered. Increased activity of these enzymes suggests injury to the hepatocytes, where the buildup of these enzymes in the plasma was caused by cell membrane leakage (Bardi et al., 1993). This results from the liver's more significant concentration of alcohol dehydrogenase, an enzyme that catalyzes alcohol conversion to its corresponding aldehyde.

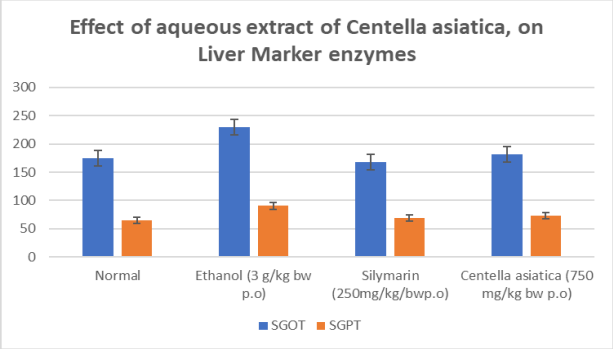


Figure 11 Effect of aqueous Extract of Centella asiatica on Marker enzymes (Liver-SGOT, SGPT) in control and ethanol-administered rats.

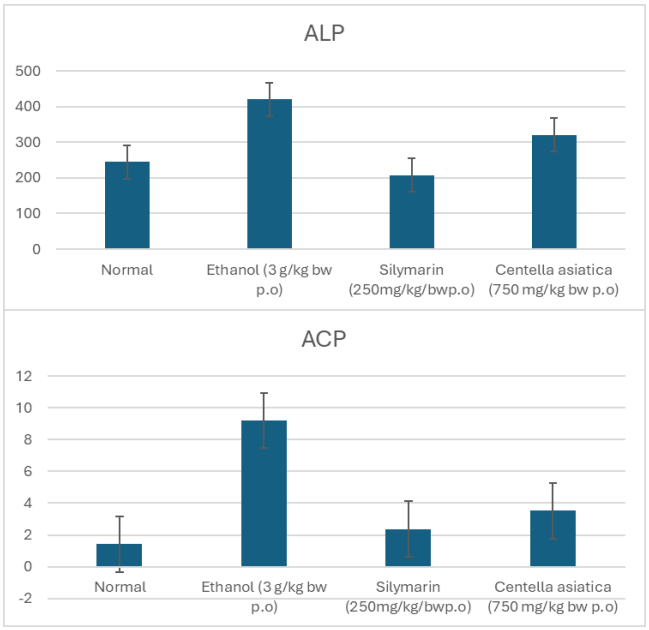


Figure 12 Effect of aqueous Extract of Centella asiatica on Marker enzymes (Liver-ALP, ACP) in control and ethanol-administered rats

Table 13 and **Figure 12** demonstrated the levels of Liver marker enzymes (ALP, ACP) of control, ethanol-induced, standard drug (Silymarin 250mg/kg bw), Centella asiatica (750 mg/kg b.w), The level of ALP in Group I was 245±3.12U/L (Positive control), and in the induced group was 420±8.25 U/L (Group II - Negative control). On administration of an aqueous Extract of Centella

Table 12 Effect of aqueous Extract of Centella asiatica on Marker enzymes (Liver-SGOT, SGPT) in control and ethanol-administered rats

Groups	Treatment	Parameters (U/L)	
		SGOT	SGPT
I	Normal	175±2.12	65.41±2.45
II	Ethanol (3 g/kg BWP.o)	230±7.25	90.21±3.14
III	Silymarin (250mg/kg/bwp.o)	168±3.85	68.36 ±2.14
IV	Centella asiatica (750 mg/kg bwp.o)	182±3.12	73.14±2.54

Values are means ± S.D.; n = 6; Group II compared with Group I; Group III & IV compared with Group II; ANOVA statistically significantly different at $P < 0.05$

Table 13 Impact of Centella asiatica aqueous extract on marker enzymes (Liver-ALP, ACP) in rats given ethanol versus control

Groups	**Treatment**	**Parameters (U/L)**	
		ALP	**ACP**
I	Normal	245±3.12	1.41±2.45
II	Ethanol (3 g/kg BWP.o)	420±8.25	9.21±3.14
III	Silymarin (250mg/kg/bwp.o)	208±4.85	2.36 ±2.14
IV	Centella asiatica (750 mg/kg bwp.o)	320±712	3.52±1.54

Values are means ± S.D.; n = 6; Group II compared with Group I; Group III & IV compared with Group II; ANOVA statistically significantly different at $P < 0.05$

asiatica (750 mg/kg b.w), the level of ALP was 320±712; in the treated Group where the level of ALP was 208±4.85U/L. Among the treated groups, the mixed formulation showed a significant decrease in the level of ALP. The level of ACP in Group I (Positive control) was 1.41±2.45 ka units, and in the induced group (Group II - Negative control) was 9.21±3.14. On administration of aqueous Extract of Centella asiatica (750 mg/kg b.w), the level of ACP was 3.52±1.54 ka units which was comparable with Silymarin treated Group where the level of ACP was 2.36 ±2.14ka units.

Effect of aqueous Extract of Centella asiatica on (Protein, Albumin, Globulin) in control and ethanol-administered rats

Table 14 Effect of aqueous Extract of Centella asiatica on (Protein, Albumin, and Globulin) in control and ethanol-administered rats

Groups	Treatment	Parameters (U/L)		
		Protein	Albumin	Globulin
I	Normal	7.3±0.4	3.7±0.2	3.5±0.3
II	Ethanol (3 g/kg BWP.o)	5.4±0.1	3.1±0.2	2.4±0.1
III	Silymarin (250mg/kg/bwp.o)	7.2±0.1	3.8±3.2	3.3±0.1
IV	Centella asiatica (750 mg/kg bwp.o)	6.6±0.2	3.3±0.1	3.2±0.1

Values are means ± S.D.; n = 6; Group II compared with Group I; Group III – Group X compared with Group II; ANOVA statistically significantly different at P < 0.05

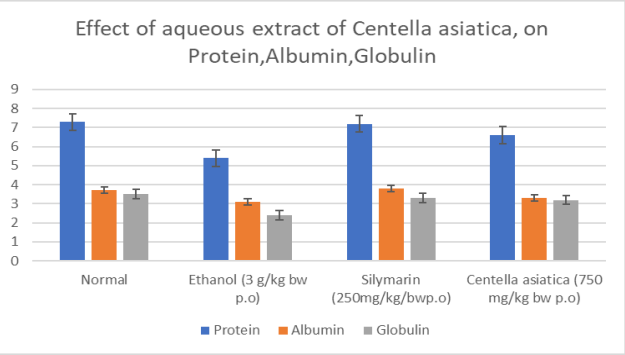


Figure 13 Effect of aqueous Extract of Centella asiatica on (Protein, Albumin, and Globulin) in control and ethanol-administered rats

Table 14 and **Figure 13** demonstrated the concentration of proteins (Total proteins, albumin, globulin) of control, ethanol-induced, standard drug (Silymarin 250mg/kg bw), Centella asiatica (750 mg/kg b.w).

The concentration of proteins (Total proteins, albumin, and globulin) in Group I (Positive control) was 7.3±0.4, 3.7±0.2, 3.5±0.3 and in ethanol-induced Group (Group II - Negative control) was 5.4±0.1 3.1±0.2, 2.4±0.1 g/dl. On administration of aqueous Extract of Centella asiatica (750 mg/kg b.w), the level of proteins (Total proteins, albumin, globulin) was 6.6±0.2, 3.3±0.1, 3.2±0.1g/dl respectively. This was comparable with the treated Group, where the level of proteins (Total proteins, albumin, and globulin) was 7.2±0.1, 3.8±3.2, 3.3±0.1. Effect of aqueous Extract of Centella asiatica on lipid profile in control and ethanol-administered rats.

Effect of aqueous Extract of Centella asiatica on lipid profile in control and ethanol-administered rats

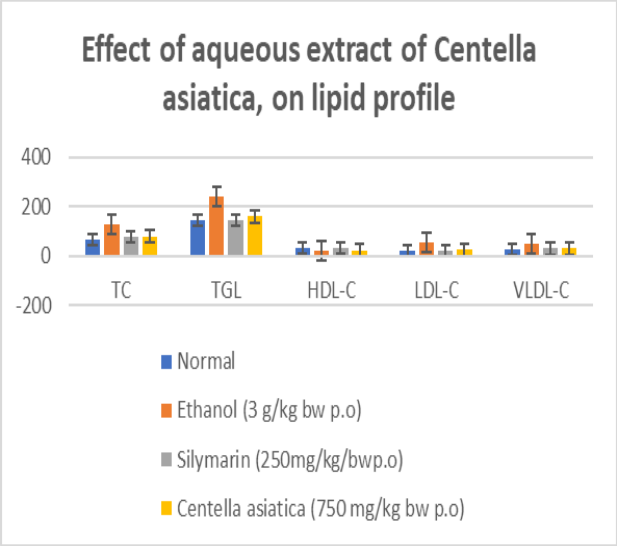


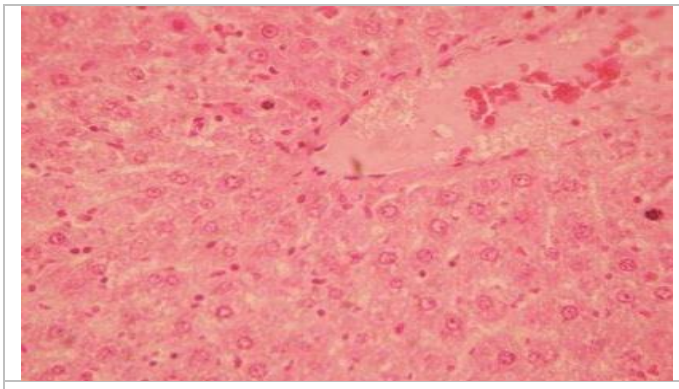
Figure 14 Effect of aqueous Extract of Centella asiatica on lipid profile in control and ethanol-administered rats

Table 15 and **Figure 14** demonstrated the Lipid profile levels of control, ethanol-induced, standard drug (Silymarin 250mg/kg bw), Centella asiatica (750 mg/kg b.w), treated rats. The level of total cholesterol in Group I was 68±1.90 mg/dl (Positive control), and in the ethanol-induced group was 127±4.00 (Group II - Negative control). On administration of an aqueous Extract of Centella asiatica (750 mg/kg b.w) comparable

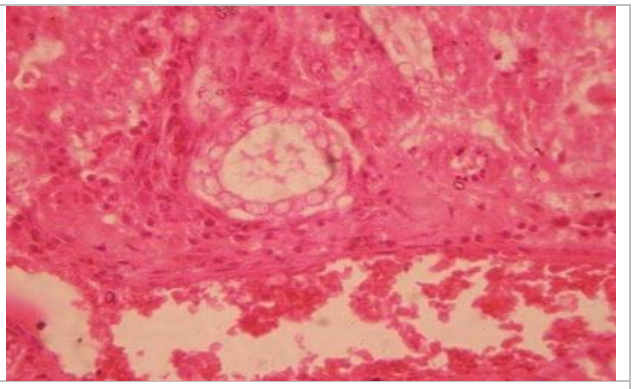
Table 15 Effect of aqueous Extract of Centella asiatica on lipid profile in control and ethanol-administered rats

Groups	Treatment	Parameters				
		TC	TGL	HDL-C	LDL-C	VLDL-C
I	Normal	68±1.90	143±1.90	33±2.68	21±2.19	29±3.52
II	Ethanol (3 g/kg bwp.o)	127±4.00	240±2.10	22±1.79	56±2.83	48±2.97
III	Silymarin (250mg/kg/bwp.o)	76±4.60	147±0.89	32±2.28	23±2.19	30±2.61
IV	Centella asiatica (750 mg/kg bwp.o)	79±3.63	160±0.82	24±2.83	26±3.74	32±2.00

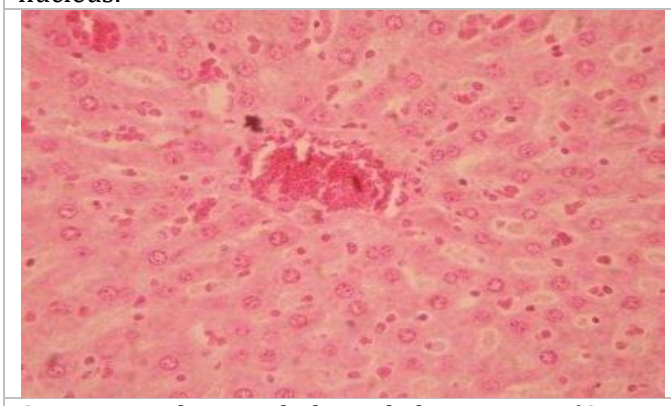
Values are means ± SD; n = 6; Group II compared with Group I; Group III & IV –compared with Group II; ANOVA statistically significantly different at P < 0.05



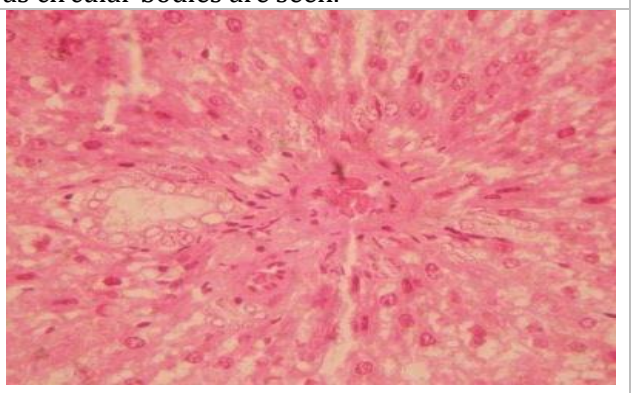
Group I: In histopathological observations (Group I), the liver of rat stained with hematoxylin and eosin showed the normal architecture with central vein and radially arranged hepatic cells with distinct nucleus.



Group II: In histopathological observations (Group II), the liver of rat induced with ethanol stained with hematoxylin and eosin showed clear dilatation of portal vein, lipid accumulation as circular bodies are seen.



Group III: In histopathological observations (Group III), the liver of rat induced with ethanol treated with Atenolol stained with hematoxylin and eosin showed prominent infiltration of RBC at the central vein region.



Group IV: In histopathological observations (Group VI), the liver of rat induced with ethanol and treated with Centella asiatica (750 mg/kg bwp.o) showed elongated portal vein at the portal triad region with extensive Ito cell formation.

Figure 15 Histopathological observation of the Liver of Ethanol Induced hypertensive rats

with Silymarin treated Group where the total cholesterol level was 76±4.60mg/dl. The level of triglyceride in Group I was 143±1.90 mg/dl (Positive control), and in the induced group was 240±2.10 (Group II - Negative control). On administration of an aqueous Extract of Centella asiatica (750 mg/kg b.w), the level of triglyceride was 160±0.. which was comparable with the Silymarin-treated Group, where the level of triglyceride was 147±0.89 mg/dl. The level of HDL in Group I was 33±2.68 mg/dl (Positive control), and in the ethanol-induced group was

22±1.79 (Group II - Negative control). On administration of an aqueous Extract of Centella asiatica (750 mg/kg b.w), similar to the Silymarin treated Group where the level of HDL was 32±2.28 mg/dl.

The level of LDL in Group I was 21±2.19 mg/dl (Positive control), and in the induced group was 56±2.83 (Group II - Negative control). On administration of an aqueous Extract of Centella asiatica (750 mg/kg b.w), the level of LDL was 6±3.74. Which was similar to the treated Group, where the level of LDL was 23±2.19 mg/dl.

The level of VLDL in Group I was 29±3.52 mg/dl (Positive control), and in the ethanol-induced group was 48±2.97 (Group II - Negative control). On administration of an aqueous Extract of Centella asiatica (750 mg/kg b.w), the level of VLDL was 32±2.00 when compared to ethanol-induced rats. This was similar to the treated Group, where the level of VLDL was 32±2.28 mg/dl.

CONCLUSION

Nowadays, lifestyle and food habits have resulted in many complaints, which is an increasing interest in identifying natural phytochemical compounds from plant species that can be used as medicines without significant side effects. Herbal medicines are multicomponent mixtures of organic and inorganic compounds, and their chemical constituents possess a pharmacological and antioxidant potential that could have the ability to protect vital organs from several ailments with less or no toxic Effect. The present study aimed to evaluate the pharmacological and antioxidant activity of well known medicinal plant Centella asiatica, which was extracted traditionally. The natural antioxidants present in the aqueous plant extracts ameliorate liver and heart damage by consuming ethanol. Hence, this study concluded that the aqueous Extract of Centella asiatica profoundly protected the vital organs. These findings support the fact that the active principles of Centella Asiatica significantly contributed to the authenticity of the development of new drugs that could alleviate society from many diseases due to oxidative stress by alcohol consumption. It is essential to evaluate the pharmacological properties, and the experimentation would be necessary to standardize and elucidate the exact mechanism of action and toxicity studies of the bioactive compounds present in aqueous Extract to be formulated as protective drugs to treat the alcoholics without any side effects.

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Conflict of Interest

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