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Research Article

Assessment of Hepatoprotective Activity of *Hyptis capitata* Jacq. Against Oxidative Stress Induced by H₂O₂

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ABSTRACT

Medicinal plants occupy a key position in maintaining the health of the human population. *Hyptis capitata* Jacq. is an exotic ethnomedicinal plant of Lamiaceae possessing immense potential. The present study was carried out to assess the *in vitro* hepatoprotective activity of methanolic leaf extract of *H. capitata* against H₂O₂ induced oxidative stress in HepG2 cell lines. Total antioxidant capacity and Superoxide radical scavenging activity of the extract were also performed. The extract displayed considerable free radical scavenging activity ($P < 0.01$). MTT assay was used to assess the *in vitro* hepatoprotective activity in HepG2 cell lines. The extract displayed concentration-dependent cell viability with maximum protection ($63.97 \pm 1.23\%$) in H₂O₂ induced HepG2 cell lines at $100 \mu\text{gml}^{-1}$. *H. capitata* is a promising herb with significant antioxidant and hepatoprotective potential.

INTRODUCTION

The plant kingdom harbors many medicinal plants, which are the treasure house of bioactive chemical constituents, making them a rich source of potential drugs.^[1] Nowadays, increasing attention is given to traditionally important medicinal plants to solve health care problems. World Health Organisation estimated that 80% of the population of the developing countries relies on plant-based drugs for their primary health care. The significance of naturally derived drugs from medicinal plants is increasingly recognized, and the public confidence in their use is constantly strengthened.^[2] The knowledge of traditional medicine is essential in developing pharmacologically active ingredients leading to active principles. The ample usage of herbal medicines as nutraceuticals continues

to expand rapidly across the world. Many people refer to these products to treat various health challenges in health care.^[3]

Liver diseases have become one of the major causes of morbidity and mortality in the human population, in which drug abuse is the major contributing factor.^[4] World Health Organisation (WHO), 2014 published that about 2.4 million deaths were reported yearly due to liver diseases, of which about 800 thousand deaths were linked to cirrhosis. The liver cells become damaged due to excessive alcohol consumption, toxic substances like thioacetamide (TAA), abuse of certain drugs such as paracetamol, chemotherapeutic drugs like carbon tetrachloride (CCl₄), and some viral infections (Hepatitis).^[5] The free radicals damage the membrane lipids, proteins, and nucleic

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acids, leading to serious pathological conditions such as atherosclerosis, diabetes mellitus, damage of kidneys and lungs, liver disorders, cancer, inflammatory diseases, and neurodegeneration.^[6] In this context, Constantin *et al.* (1990)^[7] opined that the free radical scavenging mechanisms play an important role in inhibiting lipid peroxidation and protection of liver cells. Ajiboye *et al.* 2014^[8] reported that the naturally-derived antioxidant agents such as phenols and flavonoids may defend effectively against the free radicals which generate liver injuries. Therefore, the researchers focus on investigating effective phytochemicals that have the potential to protect the liver from free radicals and drugs. Moreover, the negative impact of synthetic hepatoprotective medicines could be solved by natural remedies because of less toxicity and cost-effectiveness.

Hyptis capitata Jacq. of Lamiaceae is a slightly aromatic perennial herb commonly called knob weed and ethnomedicinally important plant. The traditional healers of tribal communities of the Philippines and Indonesia use root stem and leave of *H. capitata* for health care. The plant's curative properties include remedies for cough, fever, inflammation on wounds, toothache, and malaria. The scientific validation of the therapeutic potential of the herb is rather meager. So, the present study aimed to investigate the antioxidant potential and hepatoprotective activity by *In vitro* models.

MATERIALS AND METHODS

Plant Material

Hyptis capitata Jacq. was collected from the natural habitat of Thiruvananthapuram district, Kerala. The plant was identified and authenticated by the Department of Botany, University of Kerala, and the voucher specimen (KUBH-6166) has been deposited in the herbarium. Fresh leaves were collected, washed thoroughly, shade dried, and powdered.

Extraction

About 50 gm dried leaf powder was extracted with methanol by the Soxhlet apparatus for 48 hrs to 72 hours. The extract was filtered through Whatman No.1 filter paper and evaporated to dryness at 55–60°C.^[9] The solidified extract was stored in an airtight container for further analysis.

Antioxidant Activity

Total antioxidant capacity and superoxide anion radical scavenging assay was conducted on methanolic leaf extract of *H. capitata* to determine the antioxidant potential.

(i) Total Antioxidant Capacity Assay

The total antioxidant capacity of the extract was evaluated by the phospho-molybdenum method, according to the procedure described by Prieto *et al.*^[10] One mg of

extract was combined with 3 mL of reagent solution (0.6 M sulfuric acid, 28 mM sodium phosphate, and 4 mM ammonium molybdate). The reaction solution tubes were incubated at 95°C for 90 minutes. The absorbance of the solution was measured at 695 nm using a UV-VIS spectrophotometer against methanol as blank after cooling to room temperature. The total antioxidant activity is expressed as the number of gram equivalent of ascorbic acid.

(ii) Superoxide anion Radical Scavenging Activity

The superoxide radical scavenging activity of the extract was measured according to Nishikimi *et al.*^[11] In this assay, the superoxide radicals were generated in 3ml of sodium phosphate buffer (100 mM, PH 7.4) containing 1ml of NBT 150 µM (Nitroblue tetrazolium) solution, 1-mL of NADH (468 µM) solution, and different concentrations of the extracted sample 25, 50, 75 and 100 µg mL⁻¹ in DMSO. The reaction was started by adding 1 mL of 60 µM PMS (Phenazine methosulphate) solution to the mixture. The reaction mixture was incubated at 25°C for 5 minutes, and the absorbance was measured at 560 nm against a corresponding blank solution. Ascorbic acid was used as a standard, a decrease in the extent of NBT reduction, measured by the absorbance of the reaction mixture, correlated with the superoxide radical scavenging activity of the extract. The percentage of superoxide radical scavenging was calculated using the following equation. The antioxidant activity of the extracts was expressed as IC₅₀ value.

$$\text{Percentage of Inhibition} = \frac{(\text{OD of control} - \text{OD of Test})}{\text{OD of control}} \times 100$$

Hepatoprotective Assay

Methanolic leaf extract of *H. capitata* was subjected for the study of hepatoprotective activity followed by Siddiqui *et al.*^[12] and was evaluated on HepG2 cell line initially procured from National Centre for Cell Sciences (NCCS), Pune, India. The confluent HepG2 cells were cultured with growth media, Dulbecco's Modified Eagles Medium (DMEM), and Fetal Bovine Serum (FBS) at a density of 5×10^4 cells/well were incubated overnight at 37°C in a humidified 5% CO₂ incubator. After incubation, cells were treated with varying concentrations of the extract (25, 50, 100 µg/mL) and incubated for 24 Hrs, thereafter with; IC₅₀ concentration of H₂O₂ (82 µM) as hepatotoxicant was added and incubated for further 24 hrs. The treated cells were reconstituted with Phosphate Buffered Saline (PBS) and incubated with MTT ((3-(4,5 dimethylthiazol -2-yl)-2-5- diphenyl tetrazolium bromide). After the incubation period, the supernatant was removed, and dimethyl sulphoxide (DMSO) was added in order to solubilize the formazan crystals. The absorbance values were measured at a wavelength of 570 nm against a control. Untreated cells were kept as control, and the percentage of cell viability in treated cells was calculated. The microscopic observation



of cell lines was done by an inverted phase-contrast microscope and was recorded as images.

$$\text{Percentage of Viability} = (\text{OD of Test/OD of Control}) \times 100$$

STATISTICAL ANALYSIS

Statistical analysis was done by using Graph Pad Instat DTCG. Results of different treatments were analyzed by one-way Analysis of Variance (ANOVA). Tukey- Kramer Multiple Comparison Test was used to determine the level of significance for the individual experimental conditions; $p < 0.01$ and $p < 0.001$ were regarded as significant. The experiments were conducted in triplicate, and the mean and standard deviation was computed.

RESULTS

Antioxidant Activity

Total Antioxidant Capacity

The total antioxidant capacity of methanolic leaf extract of *H. capitata* has been evaluated by the routinely used Phosphomolybdenum method. This assay is based on the reduction degree of MO^{4+} to MO^{5+} by the antioxidants and subsequent formation of green MO^{5+} complex in acidic p^{H} conditions. The total antioxidant capacity of the methanolic leaf extract of *H. capitata* was found to be $131.02 \pm 4.06 \mu\text{g mg}^{-1}$ of ascorbic acid equivalents.

Superoxide anion Radical Scavenging Activity

In this assay, the superoxide anions generated in the riboflavin illuminated the extract's antioxidants have scavenged the NBT system. The effectiveness of scavenging potential of the extract by the inhibition of the formation of blue-colored formazan could be monitored spectrophotometrically at 560 nm. The superoxide anion radical scavenging activity of methanolic leaf extract

Table 1: Superoxide Anion Scavenging Assay in Methanolic Leaf Extract of *Hyptis capitata* Jacq.

Samples	Concentration ($\mu\text{g ml}^{-1}$)	% of Inhibition
Standard	20	$47.8 \pm 0.105^{***}$
Ascorbic acid	40	$52.7 \pm 0.406^{***}$
	60	$59.12 \pm 0.021^{***}$
	80	$61.3 \pm 0.180^{***}$
	100	$64.5 \pm 0.100^{***}$
Methanolic Leaf Extract of <i>H. capitata</i>	25	$17.73 \pm 0.010^{**}$
	50	$31.18 \pm 0.005^{**}$
	75	$44.61 \pm 0.01^{**}$
	100	$53.75 \pm 0.005^{**}$

Each value represents the mean $\pm$ SD of triplicates and the superscript represents the level of significance compared to control value (control-no activity) *** significant at $P < 0.001$, ** significant at $P < 0.01$ (according to Tukey-Kramer Multiple Comparison test)

of *H. capitata* was presented in Table 1. The scavenging of superoxide anion radical by the extract increased remarkably with an increase in the concentration of extract. The maximum percentage of inhibition of superoxide radical was $53.75 \pm 0.005\%$ and $64.5 \pm 0.100\%$ in methanolic leaf extract and standard ascorbic acid respectively at a concentration of $100 \mu\text{g mL}^{-1}$. The percentage of inhibition against the concentration was statistically significant for both extract and ascorbic acid ($p < 0.01$ and $p < 0.001$). The IC_{50} values obtained were $88.54 \mu\text{g mL}^{-1}$ and $26.17 \pm 0.165 \mu\text{g mL}^{-1}$ for methanolic leaf extract of *H. capitata* and standard ascorbic acid, respectively. The methanolic leaf extract of *H. capitata* showed considerable superoxide scavenging activity.

Hepatoprotective Activity

The hepatoprotective activity of methanolic leaf extract of *H. capitata* denoted the protection of HepG2 cells from H_2O_2 induced cell damage. After that, the cell viability or degree of protection was determined by MTT assay. The effect of methanolic leaf extract of *H. capitata* on HepG2 cells against H_2O_2 toxicity was presented in Fig. 1. The untreated cell lines (control) showed 100% cell protection, while the treated samples exhibited a noticeable hepatoprotective activity. The percentage of cell viability/hepatoprotection such as 51.62 ± 2.199 , 58.73 ± 1.39 and $63.97 \pm 1.23\%$ was registered in a dose-dependent manner for the sample concentrations 25, 50 and $100 \mu\text{g mL}^{-1}$ respectively. A significant hepatoprotection was displayed at a concentration of $100 \mu\text{g mL}^{-1}$ ($63.97 \pm 1.23\%$) as compared to lower concentrations.

The cellular morphology of treated and control HepG2 cell lines was visualized by a phase-contrast microscope and displayed in Fig. 2. The normal morphology and cell adherence of HepG2 cell lines were found to be reduced on exposure to H_2O_2 , while no change was observed in control. Most of the exposed HepG2 cell lines lost their typical morphology and appeared very smaller in size.

DISCUSSION

Generally, the consistent formation of free radicals viz. Reactive oxygen species (ROS), hydroxyl radicals, superoxide anions, hydrogen peroxide, and singlet oxygen

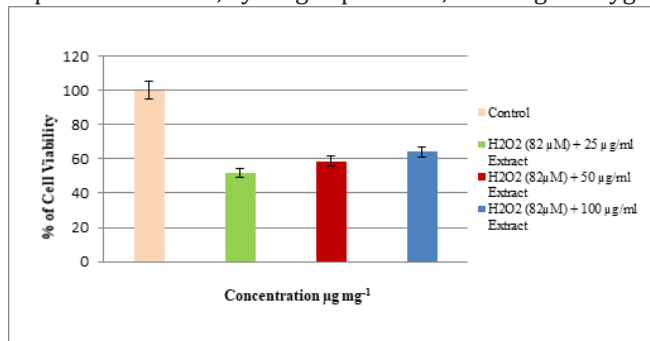


Fig. 1: Hepatoprotective Effect of Methanolic Leaf Extract of *Hyptis capitata* Jacq. on H_2O_2 Induced HepG2 Cell Lines

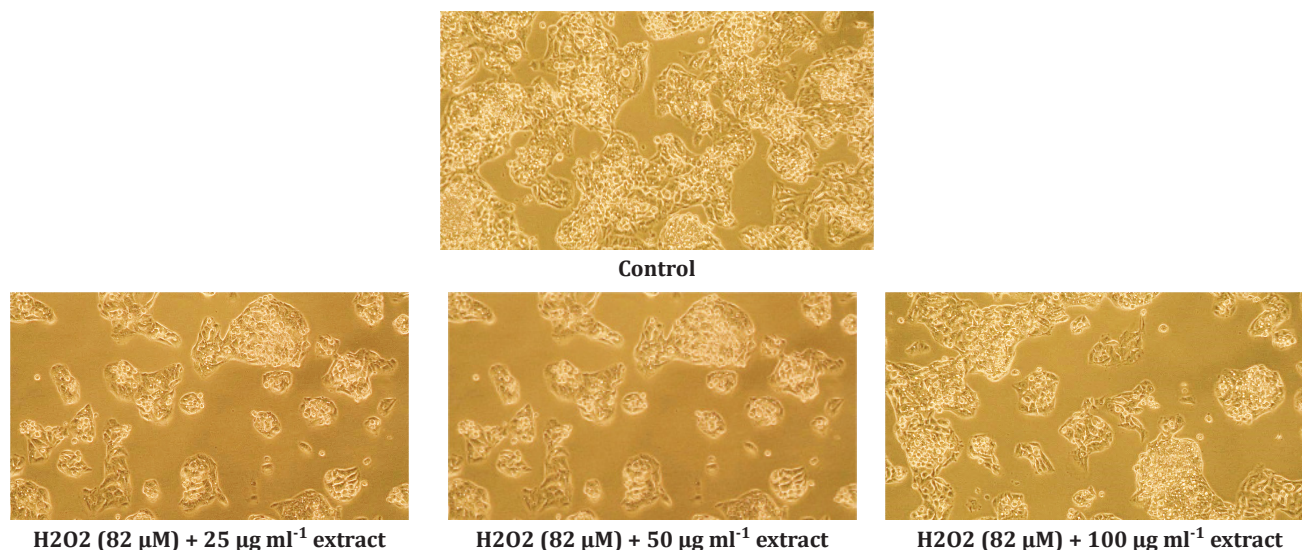


Fig. 2: Effect of Methanolic Leaf Extract of *Hyptis capitata* Jacq. on H_2O_2 Induced HepG2 cell line

in the living system develop oxidative stress and causes huge damage to the tissues, cells, and organs, which may lead to various illnesses, including cardiovascular diseases, degenerative disorders, inflammation, anemia, and cancer.^[13] The plant-derived antioxidants nutraceuticals scavenge free radicals and modulate oxidative stress-related degenerative effects.^[14] Significant radical scavenging activity was exhibited by the methanolic leaf extract of *H. capitata* in the *in vitro* models such as total antioxidant capacity assay and superoxide anion scavenging assay. The total antioxidant capacity assay is a unique assay for determining the reducing efficiency of antioxidants present in the crude extract. The antioxidant capacity assay was substantially high, indicating the overall antioxidant potential according to water-soluble and fat-soluble antioxidants. Superoxide anion has a significant role in producing other reactive oxygen species such as hydrogen peroxide, hydroxyl radicals, and singlet oxygen which adversely affects the biologically important macromolecules like protein, lipid, and DNA.^[15] The present study revealed a remarkable superoxide anion scavenging potential. It inferred that methanolic leaf extract of *H. capitata* was able to protect the biological system from oxidative stress by inhibiting lipid peroxidation. The observed antioxidant property of the methanolic leaf extract of *H. capitata* may be due to an appreciable amount of phenols and flavonoids.^[16] Francis and Andrew (2010)^[17] reported IC_{50} value $82.2 \mu g ml^{-1}$ in *Ocimum gratissimum*, which remained in agreement with the values in the extract of *H. Capitata* supporting its superoxide radical scavenging ability.

The liver is the most important indispensable organ in the body involved in the metabolism of lipids, carbohydrates, and proteins, as well as the excretion of waste materials from the body.^[18] It is endowed with metabolic and detoxification capability in the human

body. It is normally exposed to several endogenous and xenobiotic agents and a myriad of intermediate end products causing hepatocellular death leading to liver diseases.^[19] Moreover, the accumulation of many free radicals in the human body causes oxidative stress, which leads to liver damage. An antioxidative agent offers resistance to cell and prevents diseases through scavenging such free radicals by hindering the lipid peroxidation and many other mechanisms.^[20] In India, herbal-based therapeutics have been used for liver diseases. Some of the best known plant-derived isolated compounds with good hepatoprotective potential are andrographolide (*Andrographis paniculata*),^[21] asisaticoside (*Hydrocotyle sibthorpioides*),^[22] carnosic acid (*Romarinus officinalis*),^[23] curcumin (*Curcuma longa*),^[24] glycyrrhizin (*Glycyrrhizin glabra*),^[25] phyllanthin (*Phyllanthus amarus*).^[26] The phytochemicals such as alkaloids, coumarins, flavonoids, glycosides, phenols, saponins, and terpenoids have been reported as effective hepatoprotective compounds.^[27] These phytocompounds were also detected and reported in methanolic leaf extract of *H. capitata*,^[28] which may ascertain the significant hepatoprotective activity of methanolic leaf extract of *H. capitata* against H_2O_2 induced toxicity in HepG2 cell lines. The present study highlighted that the methanolic leaf extract of *H. capitata* can protect the hepatocytes from oxidative stress through superoxide anion radical scavenging, thereby inhibiting lipid peroxidation.

Hence, the present investigation revealed that the ethnomedicinal herb *H. capitata* offered antioxidant and hepatoprotective properties against oxidative stress-induced hepatotoxicity, which may validate the folk information on the therapeutic use by *in vitro* model system. However, further research is warranted through *in vivo* animal models to confirm the therapeutic property of the herb.



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