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In-Vivo Antioxidant activity of ethanolic extract of *Mentha pulegium* leaf against CCl4 induced toxicity in rats

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ABSTRACT

Objective: To evaluate the *in-vivo* antioxidant potential of ethanolic extract of *Mentha Pulegium* against CCl4 induced toxicity in rats. **Methods:** Animals were treated with plant extract for 7 days and then toxicity was induced with a single CCl4 intraperitoneal injection. Pre-treatment with 600 mg/kg (p.o.) of ethanolic extract of *Mentha Pulegium* improved the glutathione, SOD, catalase, and peroxidase levels significantly as compared to control group. **Results:** The present studies revealed that *Mentha Pulegium* has significant *in-vivo* antioxidant activity and can be used to protect tissue from oxidative stress. The result showed that the activities of glutathione, SOD, catalase and peroxidase in group treated with CCl_4 declined significantly than that of normal group. **Conclusion:** Ethanolic extract of *Mentha Pulegium* in the dose of 600 mg/kg, p.o., has improved the glutathione, SOD, catalase, and peroxidase levels significantly, which were comparable with Liv 52. Based on this study we conclude that Ethanolic extract of *Mentha Pulegium* possesses in vivo antioxidant activity and can be employed in protecting tissue from oxidative stress.

1. Introduction

Mentha Pulegium (Lamiaceae) a common garden plant in tropical countries has been used as a traditional medicine. Plants are well known as a major source of modern medicines. From ancient times, humans have utilized plants for the treatment or prevention of diseases, leading to the dawn of traditional medicine. *Mentha Pulegium* is one of the genera that are used in Chinese, Ayurvedic and Thai traditional medicine for the treatment of fever, pain and dysentery [1, 2]. Literature reveals that, the carbonyl groups are responsible for free radical scavenging activity [3]. Free radicals are atoms or groups of atoms with an odd number of electrons and can be formed when oxygen interacts with certain molecules. To prevent free radical damage, the body has a defense system of antioxidants [4]. Antioxidants are able to give free radicals, which becomes a companion to their unpaired electron, thus eliminating the threat of gene alteration leading to cancer. Medicinal plants have attracted attention of not only professionals from various systems of medicines, but also the scientific community belonging to different disciplines [5, 6]. In recent years, these have been a great interest in herbal remedies for the treatment of number of ailments. In continuation of search in potential free radical scavenging agents [7] the present investigation was aimed to determine antioxidant activity of *Mentha Pulegium* leave (Linn.) properties help in strengthening the immune system of the body which helps to overcome cancer.

The herb is antiseptic, antispasmodic, carminative,

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diaphoretic, emmenagogue, sedative and stimulant. A tea made from the leaves has traditionally been used in the treatment of fevers, headaches, minor respiratory infections, digestive disorders, menstrual complaints and various minor ailments. It is occasionally used as a treatment for intestinal worms. The basic components, among the identified 21, were pulegone (42.9–45.4%), piperitenone (21.7–23.1%) and isomenthone (11.3–12.8%). The present study was therefore carried out to evaluate the claimed antioxidant effect of *Mentha pulegium* leaves using different aspects.

The antioxidant activity of Du-Zhong (Eucomnia ulmoides) [8], ear mushrooms and anise (Pimpenella anisum L.) seed were found to correlate with the phenolic compounds. Studies on local plants such as turmeric (Curcuma domestica), betel leaf (Piper betel), pandan leaf (Panadanus odorus), asam gelugur (Garnicia atroviridis), mengkudu (Morinda citrifolia), pegaga (Centella asiatica), ginger (Zingiber officinale), cassava shoot (Manihot asculenta), kesum (Polygonum minus) and selom (Oenathe javanica) also exhibit good antioxidant activity. The antioxidative properties of some vegetables and fruits are partly due to the low molecular weight which is known to be potent as antioxidants.

2. Material and methods

2.1 Collection of plant

Leaves of *Mentha Pulegium* (Linn.) were collected from Indore MP and voucher specimen has been deposited. The authentication was done by Prof. S. R. Upadhyaya (Professor, Govt. Post graduate College, Indore) MP INDIA.

2.2 Preparation of Extracts

The leaves of *Mentha Pulegium* were collected and shade dried. The dried leaves were coarse powdered and the powder was packed in to soxhlet column and extracted with ethanol (64.5 – 65.5℃). The extract was concentrated under reduced pressure (bath temp 50℃). The dried extracts were stored in airtight container.

2.3 Preliminary Phytochemical Screening

The preliminary phytochemical screening was carried out with ethanolic extracts of *Mentha Pulegium* leaves for the detection of various phytochemicals. Tests for common phytochemicals were carried out by standard methods [9].

2. 4 Animals

Albino rats of Wister strain, weighing 100–150 g, kept on normal diet (Ashirwad Industries, Punjab) water ad libitum, were divided into six groups of six animals each. Before starting the experiment, permission from the Institutional Animal Ethics Committee was obtained.

2.5 Acute toxicity test

Acute toxicity tests were performed according to OECD – 423 guidelines [10]. Swiss mice (n = 6) of either sex selected by random sampling technique were employed in this study. The animals were fasted for 4 h with free access to water only. The ethanolic extract of *Mentha Pulegium* suspended in normal saline: Tween 80 (95:5) was administered orally at a dose of 5 mg/kg initially and mortality was observed for 3 days. The mortality was observed in 5/6 or 6/6 animals, and then the dose administered was considered as toxic dose. However, the mortality was observed in less than four mice, out of six animals then the same dose was repeated again to confirm the toxic effect. The mortality was not observed, the procedure was then repeated with higher doses such as 100, 300 and 1500 mg/kg.

2.6 Experimental

Group-I animals served as normal control, treated with vehicle (gum acacia 3% solution). Group-II animals served as toxic control, treated with CCl_4 in a single dose of 1.5 ml/kg, i.p., to produce acute toxicity. Group III served as a standard group, and was administered Liv-52 in a dose of 56 mg/kg, *p.o.* Group-IV, V and -VI animals were treated with daily doses of 200, 400 and 600 mg/kg, p.o., respectively, of ethanolic extract of *Mentha Pulegium* for 7 days. The animals of Groups III-VI were given single dose of CCl4, 1.5 ml/kg, i.p., 6 h after the last treatment. On day 8 the rats were sacrificed by carotid bleeding and liver was rapidly excised, rinsed in ice-cold saline, and a 10% w/v homogenate was prepared using 0.15M KCI, centrifuged at 800 rpm for 10 min at 4℃. The supernatant obtained was used for the estimation of catalase, peroxidase, and other enzymes. Further, the homogenate was centrifuged at 1000 rpm for 20 min at 4℃ and the supernatant was used for biochemical estimation.

2.7 Biochemical estimation

2.7.1 Estimation of glutathione

Glutathione was estimated using Ellman's reagent (5, 5-dithiobis-(2-nitrobenzoic acid) [DTNB]). The sulphydryl groups present in glutathione forms a colored complex with DTNB, which was measured by colorimeter at 412 nm. The amount of glutathione was determined using its molar extinction

coefficient of 13600/m/cm and expressed in terms of μ mol/mg of protein [11].

2.7.2 Estimation of SOD

Estimation of SOD was done by auto oxidation of hydroxylamine at pH 10.2, which was accompanied by reduction of NBT, and the nitrite produced in the presence of EDTA was detected calorimetrically [12]. One enzymatic unit of SOD is the amount in the form of proteins present in 10% liver homogenate required to inhibit the reduction of 24 mM NBT by 50% and is expressed as units per milligram of protein.

2.7.3 Estimation of catalase

Catalase activity was estimated by determining the decomposition of H2O2 at 240 nm in an assay mixture containing phosphate buffer [13]. One international unit of catalase utilized is that amount that catalyzes the decomposition of 1 mM H2O2/min/mg of protein at 37℃. Catalase activity was calculated using the millimolar extinction coefficient of 0.07 and expressed in terms of micromole per minute per milligram of protein.

2.7.4 Estimation of peroxidase

Peroxidase estimation is based on periodide formation. Periodide can be spectrophotometrically determined at 353 nm, and this is directly proportional to the peroxidase concentration in the reaction mixture containing approximate amounts of H2O2 and enzyme [14]. One unit of peroxidase activity is defined as the change in absorbance per minute and expressed in terms of units per milligram of protein.

3. Result

3.1 Phytochemicals investigation

It was found that ethanolic extract contained amino acids, steroids, flavonoid, alkaloids, glycosides and tannins.

3.2 In-vivo antioxidant activity

Phytochemical screening of the plant shows the presence of flavonoids in ethanolic extract. Acute toxicity studies revealed the non-toxic nature of the ethanolic extract of *Mentha Pulegium* up to a dose level of 2000 mg/kg body weight in rats. There was no lethality or toxic reaction found at any of the doses selected during the study. The present study was undertaken to assess the in vivo antioxidant effects of ethanolic extract of *Mentha Pulegium* on CCl_4 induced toxicity in rats. The result showed that the activities of glutathione, SOD, catalase and peroxidase in group treated with CCl_4 declined significantly than that of normal group. Co-administration of ethanolic extract of *Mentha Pulegium* at a dose of 200, 300 and 600 mg/kg for 7 days markedly prevented these CCl4 induced alteration and maintained enzymes level near to normal values. Standard (Liv 52) treated group also significantly increased the level of glutathione, SOD, Catalase and peroxidase in CCl4 induced toxic rats. (Table-1)

3.3 Statistical analysis

All analyses were run in triplicates. Data were analysed by an analysis of variance (ANOVA). Statistical analysis was performed by the Student's t-test and by ANOVA.

Table 1
Effect of ethanolic extract of *Mentha Pulegium* on biochemical parameters

Treatment	Glutathione SOD Catalase SOD(u/mg of protein) Peroxidase(μ mol/mg of protein)	Catalase(uM/min/mg of protein)	Peroxidase(u/mg of protein)
Normal control(Vehicle treated)	18.5 ± 0.094**	32±0.02**	11±0.03**	84±0.09**
Hepatotoxic Control (CCl4 Treated)	8.4 ± 0.7	19±0.01	1.1±0.01	13±0.02
Standard Liv 52	16.3 ± 0.07**	31±0.01**	8.5±0.1**	67±0.15**
EEMP 200 mg/kg	9.9 ± 0.06	23±0.02	1.6±0.2	15±0.06
EEMP 300 mg/kg	12.9 ± 0.1*	25±0.01	1.8±0.4	17±0.03
EEMP 600 mg/kg	15±0.9**	27±0.05*	5.1±0.4**	63±0.06**

EEMP: Ethanolic Extract of *Mentha pulegium*
Values are in Mean±SEM. Where* $P<0.05$ and ** $P<0.01$

4. Discussion

The traditional medicine all over the world is nowadays revalued by an extensive activity of research on different plant species and their therapeutic principles. Experimental evidence suggests that free radicals (FR) and reactive oxygen species (ROS) can be involved in a high number of diseases [15]. As plants produce a lot of antioxidants to control the oxidative stress caused by sunbeams and oxygen, they can represent a source of new compounds with antioxidant activity.

Free radicals are produced under certain environmental condition and during normal cellular function in the body.

These molecules are missing an electron, giving them an electric charge. To neutralize this charge, free radicals try to withdraw an electron from, or donate an electron to, a neighboring molecule. Other antioxidants works against the molecules that form free radicals, destroying them before they can begin the domino effect that leads to oxidative damage. For example, certain enzymes in the body, such as superoxide dismutase, work with other chemical to transfer free radical into harmless molecules. Vitamin C; an antioxidant that may prevent cataracts and cancers of the stomach; throat, mouth, and pancreas. It may also prevent the oxidation of LDL cholesterol, lowering the risk of heart disease. Literature reveals that, the carbonyl groups present in the flavonoids and phenolic compounds were responsible for antioxidant activity [15]. This investigation revealed that the *Mentha Pulegium* Linn contain pharmacologically active substance (s) such as alkaloids, glycosides, saponins, tannins, flavonoids and phenolic compounds, which are responsible for the antioxidant activity.

Ethanolic extract of *Mentha pulegium* in the dose of 600 mg/kg, p.o., has improved the glutathione, SOD, catalase, and peroxidase levels significantly, which were comparable with Liv 52. Based on this study we conclude that Ethanolic extract of *Mentha pulegium* possesses *in-vivo* antioxidant activity and may be employed in protecting tissue from oxidative stress.

Conflict of interest statement

We declare that we have no conflict of interest.

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