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Research Article

NEPHROPROTECTIVE EFFECT OF *MENTHA LONGIFOLIA* AGAINST CYCLOPHOSPHAMIDE INDUCED NEPHROTOXICITY IN RATS: A BIOCHEMICAL AND HISTOPATHOLOGICAL STUDY

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ABSTRACT

The study was designed to investigate the nephroprotective activity of ethanolic leaf extract of *Mentha longifolia* (ML) against cyclophosphamide induced nephrotoxicity in experimental rats. The Albino Wistar rats of either sex were divided in to five groups of six animals each. Group 1 and group 2 served as normal and toxic control group respectively. Other groups treated with *Mentha longifolia* leaf extract (500mg/kg, p.o) alone, low (250mg/kg, p.o) and high (500mg/kg, p.o) dose of *Mentha longifolia.* Nephrotoxicity was induced by administering cyclophosphamide (200 mg/kg, i.p.) single injection on first day of experimental period followed by administration of *Mentha longifolia* (250 and 500 mg/kg, p.o.) continuously for 10 days. The general observations and mortality were measured. Cyclophosphamide administration significantly ($p < 0.05$) decreased the levels of antioxidant markers such as superoxide dismutase (SOD), catalase (CAT) and increased lipid peroxidation (LPO). Cyclophosphamide elevated the levels of biomarker enzymes like Albumin (ALB), Creatinine (CTN) and Total protein (TP). Treatment with *Mentha longifolia* significantly ($p < 0.05$) reversed the status of serum biomarkers and oxidative enzymes in cyclophosphamide induced nephrotoxicity. Potential nephroprotective effect of *Mentha longifolia* was supported by histopathological examination that reduced severity of cellular damage of the renal epithelial cell. The biochemical and histopathology reports support the nephroprotective effect of *Mentha longifolia* which could be attributed to antioxidant activity.

Key Words: Nephroprotective, Cyclophosphamide, *Mentha longifolia*.

INTRODUCTION

Cyclophosphamide (CYP) is widely used as an antineoplastic and immunosuppressant agent.[1] It is used for the treatment of acute and chronic lymphocytic leukemias, Hodgkin's disease, multiple myeloma, soft tissue sarcomas as well as it is an immunosuppressive agent for organ transplantation, multiple sclerosis, systemic lupus erythematosus, and other benign diseases.[2] Its tumor-cell-killing activity is mainly due to its deoxyribonucleic acid (DNA) alkylation. Although it has tumor selectivity and wide spectrum of clinical uses, CYP is known to cause multiple organ toxicity.[3] CYP can cause nephrotoxicity which result in glomerular dysfunction and tubular dysfunction, glomerular proteinuria, tubular proteinuria, reduction of glomerular filtration rate, and decrease in concentration function of kidney. Excessive generation of free oxygen radicals and decrease in the antioxidant defense mechanism by CYP may be the prime reason of nephro-toxicity.[2]

Natural products and herbal medicines have been used traditionally for various ailments to avoid side effects due to treatments. Plants produce significant amount of antioxidants such as polyphenols, phenols and flavonoids. These compounds are potential chemo preventive agents, due to their hydrogen-donating and metal-chelating capacities. Moreover, some studies have shown that plant base natural products could provide protection against toxicities caused by cyclophosphamide administration.[4]

Mentha longifolia is the herb belongs to the family Lamiaceae, which is used for the treatment of bronchitis, flatulence, anorexia, ulcerative colitis and liver complaints due to their anti-inflammatory, carminative, antiemetic, diaphoretic, antispasmodic, analgesic, stimulant, emmenagogue and anti-catharral activities.[5] *Mentha longifolia* also shows the activities such as anticancer,[6] antimutagenic,[6] antihypertensive,[7] antioxidant,[8] antifungal,[5] antibacterial,[9] anti-viral,[10] anti-ulcer[11] and anticholinesterase.[12] *Mentha longifolia* contain mainly five flavanoids identified as luteolin-7-O-glycoside, luteolin-7,3-O-diglycoside, apigenin, quercetin-3-O-glycoside and kaempferol-3-O-glycoside. Among these molecules, the quercetin-3-O-glycoside had the highest antibacterial activity.[13] The other major components present are cis-piperitone epoxide, piperitenone oxide, carvone, pulegone, menthone, thymol, menthol, β-thyione, carvacrol and (E)-caryophyllene.[14]

However, there is no scientific report which indicates the protective effect of *Mentha longifolia* against CYP-induced nephrotoxicity. So, the present study was undertaken to evaluate the effect of *Mentha longifolia* against CYP-induced nephrotoxicity.

MATERIALS AND METHODS

Preparation of *Mentha longifolia* Extract

The plant of *Mentha longifolia* was collected from Riyad, KSA in the month of September 2015. The material was procured and identified by Dr. Shanavaskhan AE, scientist, king abdulaziz city for science and technology, Riyad, KSA. The leaves were cut in to small pieces and shade dried. It was then coarsely powdered and extracted using ethanol (80%) by maceration process. The obtained ethanolic extracts were filtered and evaporated by using a rotary evaporator and freeze dryer, respectively to give the crude dried extract[8]. The yield was found to be 15.38% (W/W). Extract was freshly dissolved in tween 80% before giving each dose to animals.

Experimental Animals

Healthy adult Wistar albino rats of either sex weighing 175-250 g were procured from Animal House, Shree Devi College of Pharmacy, Mangaluru. Rats were housed in polypropylene cages, maintained under standardized condition (12 h L: D cycles, 25° ± 5°C) with paddy husk bedding at the Central Animal House, Shree Devi College of Pharmacy, Mangaluru. Animals were provided with standard pellet food and had free access to purified drinking water. The guidelines of Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA), Ministry of Social Justice and Empowerment, Government of India were followed and prior permission was sought from the Institutional Animal Ethics Committee for conducting the study (SDCP/IAEC-01/2014-15).

Experimental Protocol

Animals divided into five groups of six animals each. Group 1 served as normal control and administered 1 ml per kg body weight (p.o.) of 0.5 % carboxymethyl cellulose (CMC) daily for 10 days. Group 2 served as cyclophosphamide control where animals were administered with a single dose of CYP (200 mg/kg, i.p.) on the first day of experimental period. Group 3 animals were treated with ethanol extract of *Mentha longifolia* at (500 mg/kg p.o.) alone for 10 days. Group 4 animals received single dose of CYP (200 mg/kg i.p.) on first day followed by the administration of ethanol extract (250 mg/kg) of ML continuously for 10 days. Group 5 animals were daily administered ML ethanol extract (500 mg/kg) of for 10 days immediately after a single dose of CYP (200 mg/kg i.p.) on first day.[15]

Oxidative Marker Enzymes Assay

Twenty-four hour after the last treatment, blood was collected for the separation of serum and analyzed for Albumin (ALB),[16] Creatinine (CTN)[17] and Total protein (TP).[18] Estimation of marker enzymes were done by using commercial kits with the help of semi-auto analyzer (model: Prietest touch, Robonik India PVT.LTD.).
Then the animals were sacrificed by mild ether anaesthesia. The heart tissue was homogenized with sucrose solution (0.25 M) for estimations of superoxide dismutase (SOD),[19] catalase (CAT)[20] and lipid peroxidation (LPO).[21]

Histopathological Evaluation

The kidney tissue was dissected and fixed in 10% formalin. The paraffin sections were prepared and stained with haematoxylin and eosin for the examination using the light microscope.[22]

Statistical Analysis

Results are expressed as mean ± SEM. Statistical significance was assessed using one-way analysis of variance (ANOVA) followed by Tukey-Karmer multiple comparison tests. $P < 0.05$ was considered significant.

RESULTS

Serum Enzyme Biomarkers

CYP control group demonstrated extremely significant increase in serum Albumin (ALB), Creatinine (CTN) and Total protein (TP) values compared to normal control. *Mentha longifolia* (ML, 250 mg/kg and 500mg/kg) treated rats significantly decreased the ALB, CTN and TP values in dose dependent fashion compared to CYP control group [Table 1, Figure 1,2].

Effect of SOD and Catalase

SOD and catalase activity were reduced extremely significantly in CYP control compared to normal control. Experimental groups such as *Mentha longifolia* (250 mg/kg and 500mg/kg) treated groups resulted in extremely significant improvement in SOD and catalase activity compared to CYP-treated group [Table 2, Figure 3,4].

Effect on Lipid Peroxidation

CYP control group exhibited extremely significant increase in LPO levels compared to normal control. *Mentha longifolia* treatment in a dose dependent manner demonstrated extremely significant reduction in LPO levels compared to CYP control group [Table 2, Figure 3,4].

Histopathological Observations

CYP control group showed distorted structure of rat kidney by severe necrosis of the tubules. Engorged blood vessels and areas of heamorrhage were seen, suggesting severe tubular necrosis. Thus confirming the nephrotoxic effect of CYP at the dose used. *Mentha longifolia,* provided dose dependant protection against CYP induced renal epithelial cells damage [Figure 5].

DISCUSSION

The aim of the present study was to investigate the effect of ML leaf extract against CYP-induced nephrotoxicity.

From the documented results, it can be concluded that ML leaf extract (250 and 500 mg/kg, p.o.) showed beneficial results dose dependently.

Mentha longifolia contain different flavanoids, monoterpene ketones, tannins and saponins which are responsible for different pharmacological and biological activity of these plants. Flavanoids are a class of polyphenolic substances with reported properties including free radical scavenging, inhibition of hydrolytic and oxidative enzymes and anti-inflammatory action.
Nephrotoxicity is caused due to the two active metabolites of CYP, named phosphoramide mustard and acrolein. Phosphoramide produces an antineoplastic effect and acrolein produces free radicals by interacting with body's antioxidant defense system. Lipid peroxidation is one of the main reasons of cyclophosphamide-induced toxicity, due to the production of

acrolein. Nephrotoxicity was characterized by marked reduction in SOD and Catalase and elevated lipid peroxidation level.

In our present finding, animals treated with only CYP demonstrated significant decrease in SOD, Catalase and significant increase in lipid peroxidation, which indicates the induction of renal toxicity. Prophylactic treatment with *Mentha longifolia* dose dependently increase SOD and Catalase activity and decrease lipid peroxidation level which justify it's protection.

Elevations in serum Albumin, Creatinine and Total protein level can serve as clinical indicators of poor kidney function. Acrolein produced during CYP metabolism is known to be nephrotoxic.
In this study, the animals treated with only CYP showed remarkable increase in the levels of serum markers like Albumin, Creatinine and Total protein. Prophylactic treatment with *Mentha longifolia* dose dependently decrease Albumin, Creatinine and Total protein level which justify it's protection.

Histological study in CYP induced nephrotoxicity supported the findings of other parameters analyzed in different treatment groups. For the normal kidney, occasional blood vessels were seen in both cortex and medulla. Treatment with *Mentha longifolia* dose dependently inhibited CYP induced renal damage by preventing the focal degenerative changes in the renal tubular epithelial cells. *Mentha longifolia* predominantly in higher dose (500 mg/kg) was able to retrieve the pathological changes associated with CYP in renal epithelial cell.

Table 1: Effect of *M.longifolia* on serum level of albumin, creatinine and total protein in CYP induced nephrotoxicity in rats

Treatment	Albumin (mg/dl)	Creatinine (mg/dl)	Total Protein (mg/dl)
Normal control	4.63±0.13	1.57±0.04	51.76±0.13
Cyclophosphamide (CYP) control	9.74±0.21***	4.76±0.25***	76.67±0.32***
***Mentha longifolia* (ML)**	3.74±0.12^{ns}	1.63±0.02^{ns}	51.73±0.12^{ns}
CYP + ML 250mg/kg	7.77±0.23$^{***\#\#\#}$	4.15±0.09$^{***\#}$	67.55±0.18$^{***\#\#\#}$
CYP + ML 500mg/kg	5.68±0.33$^{*\#\#\#}$	2.61±0.08$^{**\#\#\#}$	56.36±0.22$^{***\#\#\#}$

All the values are in Mean±SEM, n=6.ns= not significant, *P<0.05, **P<0.01, ***P<0.001 when compared to normal, $^{\#}$P <0.05, $^{\#\#}$P<0.01, $^{\#\#\#}$P<0.001 when compared to CYP control.

Table 2: Effect of *M.longifolia* on antioxidants SOD, Catalase and lipid peroxidation in CYP induced nephrotoxicity in rats

Treatment	SOD (Unit/mg Protein)	Catalase (Unit/mg Protein)	Lipid peroxidation (Unit/mg Protein)
Normal control	14.62±0.20	11.85±0.15	5.42±0.26
Cyclophosphamide (CYP) control	4.47±0.19***	4.67±0.14***	35.91±0.05***
***Mentha longifolia* (ML)**	15.47±0.10^{ns}	11.66±0.20^{ns}	5.49±0.26^{ns}
CYP + ML 250mg/kg	8.39±0.19$^{***\#\#\#}$	7.79±0.20$^{***\#\#\#}$	21.10±0.47$^{***\#\#\#}$
CYP + ML 500mg/kg	10.47±0.27$^{***\#\#\#}$	9.30±0.14$^{***\#\#\#}$	17.99±0.06$^{***\#\#\#}$

All the values are in Mean±SEM, n=6.ns= not significant, *P<0.05, **P<0.01, ***P<0.001 when compared to normal, $^{\#}$P <0.05, $^{\#\#}$P<0.01, $^{\#\#\#}$P<0.001 when compared to CYP control.

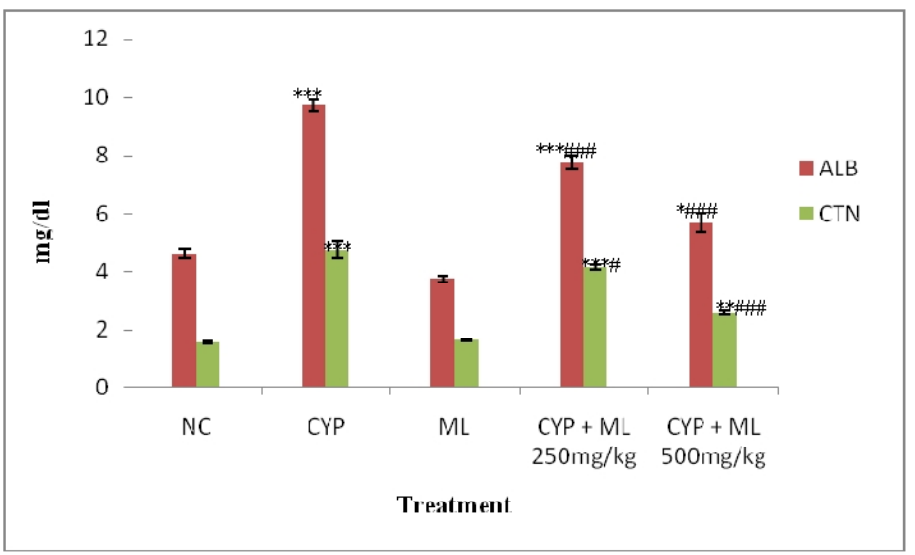


Figure 1: Effect of *M.longifolia* on serum level of Albumin and Creatinine in CYP induced nephrotoxicity in rats
All the values are in Mean±SEM, n=6.ns= not significant, *P<0.05, **P<0.01, ***P<0.001 when compared to normal, $^{\#}$P <0.05, $^{\#\#}$P<0.01, $^{\#\#\#}$P<0.001 when compared to CYP control.

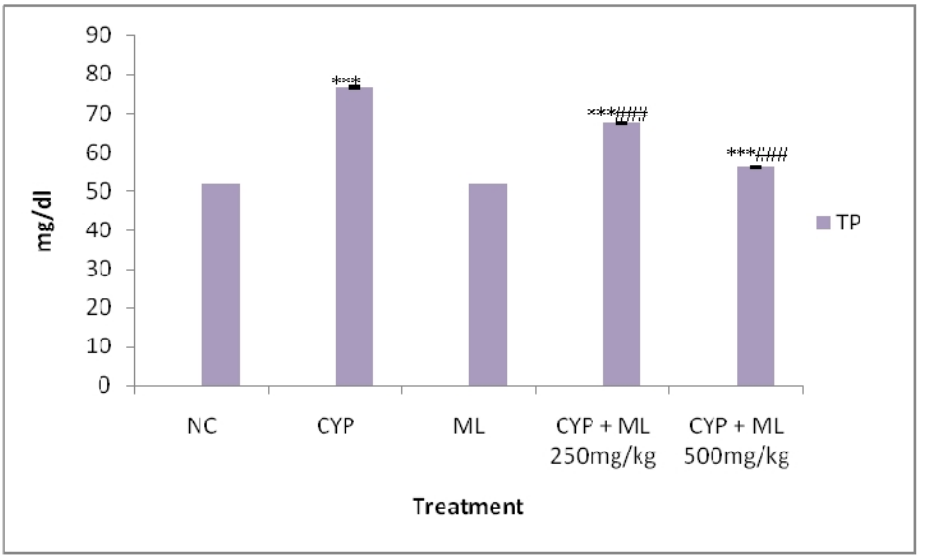


Figure 2: Effect of *M.longifolia* on serum level of Total protein in CYP induced nephrotoxicity in rats
All the values are in Mean±SEM, n=6.ns= not significant,*P<0.05,**P<0.01,***P<0.001 when compared to normal, $^{\#}$P <0.05,$^{\#\#}$P<0.01,$^{\#\#\#}$P<0.001 when compared to CYP control.

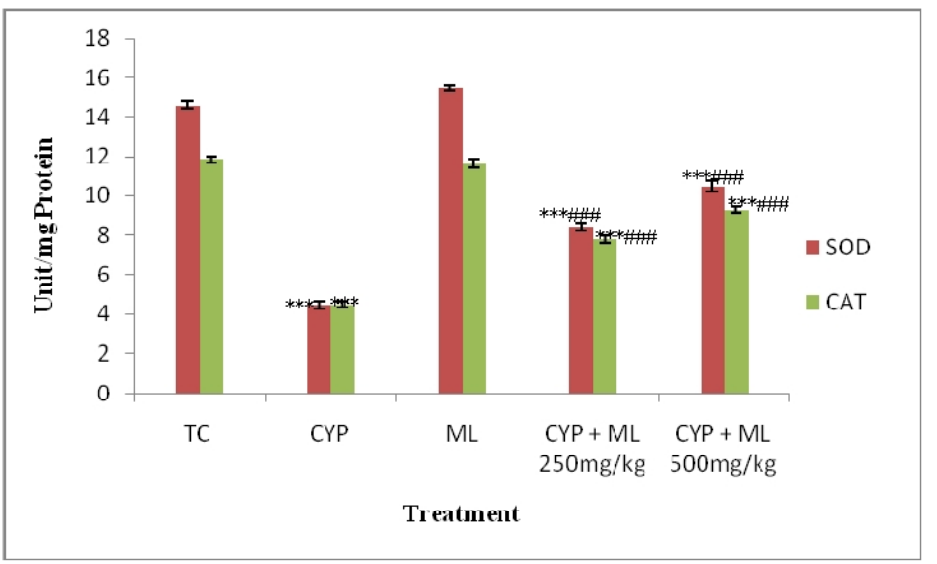


Figure 3: Effect of *M.longifolia* on antioxidants SOD and Catalase in CYP induced nephrotoxicity in rats
All the values are in Mean±SEM, n=6.ns= not significant,*P<0.05,**P<0.01,***P<0.001 when compared to normal, $^{\#}$P <0.05,$^{\#\#}$P<0.01,$^{\#\#\#}$P<0.001 when compared to CYP control.

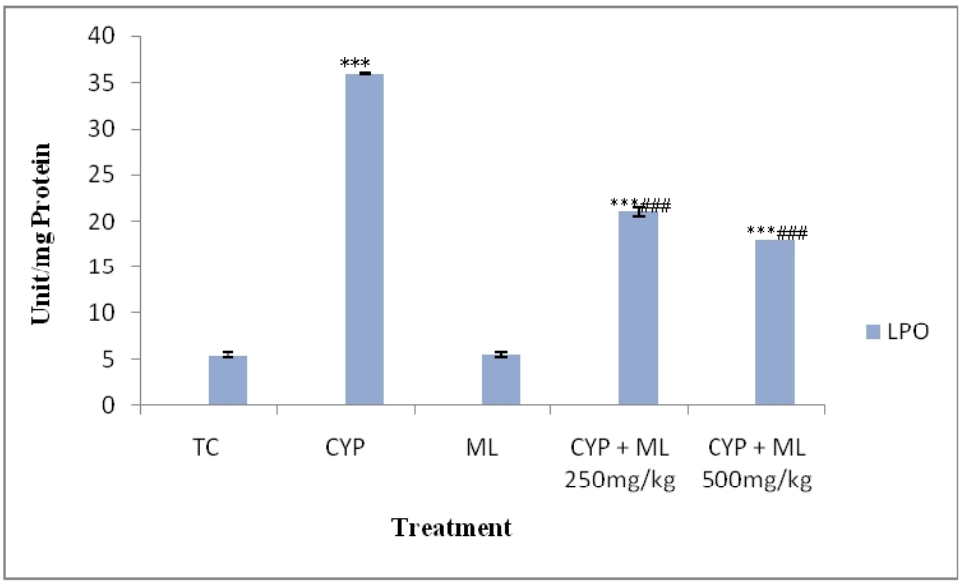
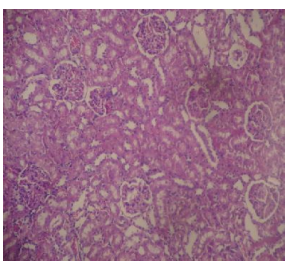
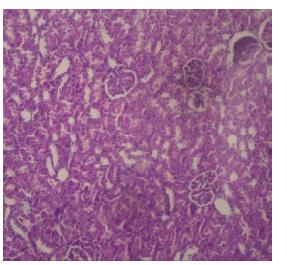


Figure 4: Effect of *M.longifolia* on antioxidant of Lipid peroxidation in CYP induced nephrotoxicity in rats

All the values are in Mean±SEM, n=6.ns= not significant, *P<0.05, **P<0.01, ***P<0.001 when compared to normal, $^{\#}$P <0.05, $^{\#\#}$P<0.01, $^{\#\#\#}$P<0.001 when compared to CYP control.



5(a)



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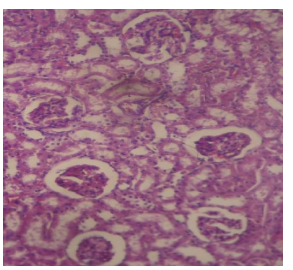

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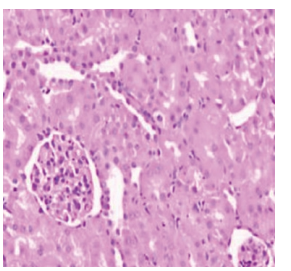

5(d)

Figure 5: Histopathological evaluation of kidney tissue in (a) normal (b) CYP treated (c) CYP+ML (250mg/kg) treated and (d) CYP+ML (500mg/ kg) treated rats

CONCLUSION

It can be concluded from the present study that CYP treatment causes pronounced oxidative stress and tissue damage in the kidney. Administration of ML extract protects the CYP induced nephrotoxicity in dose dependent manner. Biochemical and histopathological studies confirm the nephroprotective role of *Mentha longifolia*. Future studies can be carried out to establish the fact clinically.

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