

Hepato-protective potential and phytochemical screening of *Cymbopogon citratus*

Abstract

Cymbopogon citratus (Lemon grass) is a widely used herb in South Asia. The previous studies suggest that it possesses various pharmacological activities such as anti-amoebic, antibacterial, anti diarrheal, antifungal and anti-inflammatory properties. Various other effects like antimalarial, antimutagenicity, antimycobacterial, antioxidants, hypoglycemic have also been studied. The purpose of this study is to evaluate hepatoprotective activity and phytochemical screening of aqueous extract of *Cymbopogon citratus*. The aqueous extract of *Cymbopogon citratus* was administered at a dose of 200mg/kg of body weight orally for 15 days to normal healthy rabbit. The activity of extract was evaluated by determining enzyme level. The biochemical parameters, Alkaline phosphate (ALP), serum glutamic oxaloacetic transaminases (SGOT), serum glutamic pyruvic transaminase (SGPT), gamma transaminase (GT) and Total bilirubin (TB) were analyzed and the degree of improvement in these enzyme levels were evaluated. The levels of biochemical parameters were compared with Control group. The *Cymbopogon citratus* extract at a dose of 200mg/kg exhibited significant difference. Reduction in biochemical parameter (ALP, SGOT, SGPT, GT and TB) were observed. Furthermore, the photochemical profile of the extract revealed the presence of bioactive elements which may be responsible for antioxidant effect. The result suggests that *Cymbopogon citratus* possesses significant hepatoprotective activity and this may be due to presence of different bioactive components.

Keywords: cymbopogon citrates, phytochemical screening, hepatoprotective effect

Volume 3 Issue 6 - 2016

Asma Eraj, Sana Sarfaraz, Khan Usmanhany

¹Department of Pharmacology, Jinnah University for Women, Pakistan

²Research and Development Department, Pakistan

Correspondence: Sana Sarfaraz, Department of Pharmacology, Faculty of Pharmacy, Jinnah University for Women, Karachi, Pakistan, Email sana.sarfaraz@live.com

Received: November 04, 2016 | **Published:** December 15, 2016

Abbreviations: ALP, alkalinephosphate; SGOT, serum glutamic oxaloacetic transaminases; SGPT, serum glutamic pyruvic transaminase; GT, gamma transaminase; TB, total bilirubin; ROS, reactive oxygen species; DMCT, dunnett's multiple comparison test; ANOVA, analysis of variance

Introduction

Liver is a vital organ that generates reactive oxygen species that are responsible for oxidative tissue damage. Reactive oxygen species (ROS) react with cell membrane and induce lipid peroxidation that results in inflammation. It has been implicated as important pathological mediator in many clinical disorders.¹ Hepatotoxicity is one of the main reasons behind withdrawal of drug from the market. 5% of inpatient in hospitals suffer from drug associated liver disorders where as 50% of acute liver failures are result of drug-induced hepatotoxicity.²

Medicinal plants are available for treatment of hepatic disorders.³ In modern medicine there is hardly any drug available that helps in hepatic cell regeneration or protects liver from drug induced hepatotoxicity.⁴

Cymbopogon citratus (lemon grass) belonging to the family Poaceae is an aromatic perennial tall grass. It has short underground stem with ringed segments, coarse green slightly leathery leaves in dense clusters. The plant is a native herb from India and is cultivated in other tropical and subtropical countries^{5,6} and used traditionally in Indian, Chinese, and Brazilian medicines, and its oil and extract is extensively used as flavoring, scent, and medicine. The constituent obtained from *Cymbopogon citratus* is Citronelle, acts as an antihypertensive agent by inducing the vasodilatation of vascular smooth muscles.⁷ Its pharmacological action has also been reported as sedative, antispasmodic, analgesic, antipyretic and anti-inflammatory.⁸ In Ayurvedic medicine and previous literature studies

also various pharmacological activities like treatment of obesity, lipid disorders, rheumatoid arthritis, diabetes, antifungal and pneumonia has been reported. It also plays a vital role in aromatherapy.^{9,10} The previous study also reported that *Cymbopogon citratus* (lemon grass) plant generally used traditionally for the treatment of malaria and typhoid fever.¹¹ Concoction prepared from the combination of the leaves and grass of these plants have been used in the treatment of ailments like typhoid fever, stomach ache etc.¹² The medicinal plants contain valuable chemical substances that produce a definite physiological action in human body.¹³ These biological active components isolated from medicinal plant species. The most important of these bioactive constituents are alkaloids, tannins, flavonoids, phlobotannins, saponins, cardiac glycoside, citral oil, C-glycosylflavones, orientin, isoorientin, and chlorogenic acid.^{14,15}

The purpose of this study is to evaluate the Phytochemical analysis and hepatoprotective potential of Lemon grass (aqueous extract), administration against altered activities of key enzymes of Liver SGOT, SGPT, Alkaline phosphate, Gamma GT and total bilirubin in a healthy rabbit.

Methodology

Plant material collection and extraction

Lemon grass (*Cymbopogon citratus*) was purchased from local market reference no. 99. The herb was identified and authenticated by Department of Pharmacognosy Faculty of Pharmacy Jinnah University for women and was given voucher # JUW-H1003. The plant material was dried in the shade by exposing to air at (33 ± 2) °C and ground into a fine powder. The dry powder (200gm), was extracted with 800 mL of distilled water for 7 days and filtered by using Whatman filter paper no. 1. Then the concentrated extract was stored in sealed, dark glass bottles under free moisture conditions and kept frozen until use.

Phytochemical screening

The extract of *Cymbopogon citratus* was screened for the presence of carbohydrate, alkaloids, saponins and tannins by following standard procedure.¹⁶

Serum hepatospecific markers

SGPT and SGOT Evaluation: Activities of serum glutamate oxaloacetate transaminase (SGOT) and serum glutamate pyruvate transaminase (SGPT) were estimated by the method of Reitman et al.,¹⁷ 0.05ml of serum with 0.25ml of substrate (aspartate and α -ketoglutarate for SGOT; alanine and α -keto glutarate for SGPT, in phosphate buffer pH 7.4) was incubated for an hour in case of SGOT and 30min for SGPT. 0.25ml of DNPH (2,4-dinitro phenyl hydrazine) solution was added to arrest the reaction and kept for 20 min at room temperature. After incubation 1ml of 0.4N NaOH (sodium hydroxide) was added and absorbance was read at 505nm in *uv-vis* spectrophotometer. Activities were expressed as IU/L based on the method of King and Armstrong.¹⁸

Alkaline phosphatase determination: Alkaline phosphatase activity was assayed using Malloy and Evelyn.¹⁹

Total Bilirubin Estimation: Diazotised sulphonilic acid (0.25ml) reacts with bilirubin in diluted serum (0.1ml serum + 0.9ml distilled water) and forms purple colored azobilirubin, which was measured at 540nm in *uv-vis* spectrophotometer. Activities of total bilirubin were expressed as mg/dl. Serum total protein level was estimated based on the method of Gornall et al.,²⁰

Study design

Twelve healthy male rabbit were used in this study each 2-2.5kg of body weight. The rabbit were housed separately under standard condition. The study was approved by institutional Animal ethical Committee of Jinnah University for Women Ref. No: BASR/ Extr/47th Proc/June. Animal were divided into two different groups:

- a. **Group I:** Control given distilled water.
- b. **Group II:** Treated with Aqueous extract of *Cymbopogon citratus* (AqExCC). Dose of *Cymbopogon citratus* was 200mg/kg. According to rabbit weight the dose is adjusted after diluting in 8ml of distilled water. The course of treatment was for 15days. After fifteen days the blood was withdrawn by cardiac puncture allowed to clot for 45min and serum was separated by centrifugation at 2500rpm for 10min.

Statistical analysis

The experimental results were expressed as the Mean \pm SD for six animals in each group. The Biochemical parameters were analysed statistically using one-way analysis of variance (ANOVA), followed by Dunnett's multiple comparison test (DMCT). P value of < 0.05 was considered as statistically significant.

Result

Phytochemical profile

The aqueous extract of *Cymbopogon citratus* was positive for Fehling, Molish, Benedict test, Saponins and tannins shown in Table 1.

Hepatoprotective activity

A significant decrease in serum SGPT, SGOT, ALP and total bilirubin level was observed in Healthy rabbits. Treatment

with *Cymbopogon citratus* (200mg/kg p.o.) for 15days decreased the above parameters significantly ($p < 0.05$). The hepatoprotective effect at dose of 200 mg/kg was highly significantly reduced as compare to control rabbit shown in Table 2.

Table 1 Phytochemical Screening of Lemon Grass

S.no	Test	Lemon Grass
1	Carbohydrate:	
	Molish Test	+
	Iodine	—
	Fehling	+
	Benedict test	+
	Barfoed's test	-
2	Seliwanoff's test	—
	Alkaloid:	
3	Mayers test	—
	Tannins	+
4	saponins	+

Table 2 Biochemical parameter of Aqueous Extract of Lemon Grass

Groups	ALT	AST	Alkaline Phosphatase	GT	Bilirubin
Control	66 \pm 0.81	70.2 \pm 1.55	98.5 \pm 0.66	5.6 \pm 0.96	0.17 \pm 0.89
Treated	19.16 \pm 0.752***	47.8 \pm 1.16***	48.33 \pm 1.2***	3 \pm 0.89***	0.038 \pm 0.75***

N = 6 Values are Mean \pm SD using One way Anova Post Hoc analysis was done. Dunnett's test applied and P values are *** $p < 0.0001$ highly significant as compared to Control.

Discussion

The activities of the liver function enzymes are good biomarkers for evaluating the protective effect of medicinal plants.²¹ Alanine transaminase (ALT) also called serum glutamate pyruvate transaminase or alanine aminotransferase is an enzyme present in hepatocytes.²⁰ When a cell is damaged; it leaks into the blood along with other cellular contents where they can be measured. ALT rises in acute liver damage, such as viral hepatitis, drug overdoses Aspartate transaminase (AST) also called serum glutamate oxalate transaminase (SGOT) or aspartate aminotransferase is similar to ALT in that it is another enzyme associated with liver parenchymal cells.²² It is raised in acute liver damage, but it is also present in red blood cells, cardiac and skeletal muscles; therefore, not specific to the liver. The ratio of AST to ALT is sometimes useful in differentiating between causes of liver damage.¹⁴ Elevated AST levels are not specific for liver damage; and AST has also been used as a cardiac marker. Alkaline phosphatase (ALP) is an enzyme in the cells lining the biliary ducts of the liver, ALP levels rises with large bile duct obstruction, intrahepatic cholestasis or infiltrative disease of the liver. ALP is also present in the bone and placental tissue, so it is higher in growing children (as their bones are being remodeled) and the elderly patients with Paget's disease.^{23,24} The GT function in the body as a transport molecule. It plays a significant role in the drug and toxin metabolism. Increased level of GT indicates liver disorder or alcohol toxicity. The result suggests that lemon grass extract is also responsible for significant reduction in GT levels. The reduction of total bilirubin levels may not be unconnected with reduction in degradation of red cells and haemoglobin and maintenance of red cell integrity. Biochemical evaluation of medicinal plants is a common tool for the assessment of the usefulness of herbs and it is likely to reveal their potential toxicity or safety. The present study demonstrates that *C. citratus* aqueous extract has hepatoprotective effect by the significant reduction in the levels of ALT, AST, ALP, GT and bilirubin.²⁵ Many

biologically and chemically active substances have been investigated in *C. citratus*. The free radical antioxidant activity of these substances has been reported by several authors.²⁶ *Cymbopogon citratus* extract protects the integrity of the plasma membrane by reducing the leakage of serum aminotransferase and LDH. Simultaneously, it also increases the regenerative capacity of the liver by reducing the liver oxidative stress, showed by reducing biochemical profile.²⁷ The bioactive constituents of *Cymbopogon citratus* act as hepatoprotective agents because of their antioxidant properties due to presence of reducing sugar, polysaccharides and saponins and protect rabbit liver. On the basis of their results, Koh et al.,²⁸ suggested that the efficacy of the hepatoprotective effects of the *Cymbopogon citratus* extract may be largely related to the phenolic content and its free radical activity, which stabilizes the membrane and maintains the normal functioning of hepatocytes.²⁹ Phenols such as isoorientin-2-O-rhamnoside, chlorogenic acid, and caffeic acid are well-known plant antioxidants. These phenolic compounds were reported to exhibit hepatoprotective properties.^{26–29} It exhibits hepatoprotective effect on hepatocytes by reducing biochemical profile. The extracts of *Cymbopogon citratus* exerted reduction in AST, ALT, ALP direct and total bilirubin levels in the treated animals. Lemon grass has been reported to contain phytochemicals including flavonoids. Flavonoids are also reported to exhibit antioxidant activity.⁵ A phenomenon that favors 'hepatoprotective potential. Table 1 shows the phytochemical screening results besides these Rao et al.,²⁵ extracted myrcene from the species essential oil which possess anti-nociceptive effect in mice.²⁵ This antinociceptive activity of the essential oil of lemon grass was also confirmed in 2000 by Viana et al.,²⁸

In 1976, Hanson et al.,²⁷ isolated two triterpenoids- the former being ketone.²⁷ *Cymbopogon* is denominated and previously isolated and reported by Crawford and other compound isolated was alcohol derived namely Cimbopogonol. It also suggested that the structural relationship verified between the cimbopogonol and the cimbopogone recommends the possibility that the cimbopogone isn't a natural product.³⁰ Ketonic compound (ionones) were also isolated by Faruq et al.,³⁰ The citral is present abundantly in Lemon grass about 30–93.74% and mixture of aldehyde neral and geranial has also been reported by Silva and Bauer et al.,³¹ Mican et al.,³² described the isolation of the flavanoids myrcene, quercetin, kaempferol and apigenine and Faruq et al.,³⁰ obtained the phenolic compounds elemicin, catechol and hydroquinone.^{29,31}

Conclusion

In conclusion, the current results demonstrate that *Cymbopogon citratus* has a potent hepatoprotective effect in healthy rabbit. *Cymbopogon citratus* treatment significantly reduced liver enzyme activities. The aqueous extract could be claimed for prevention or early treatment of patients suffering from hepatic disorder. Furthermore investigations are needed to isolate, identify and explore the functional photochemical of *Cymbopogon citratus*.

Acknowledgments

None.

Conflicts of interest

Author declares there are no conflicts of interest.

Funding

None.

References

1. Dash DK, Yeligar VC, Nayak SS, et al. Evaluation of hepatoprotective and antioxidant activity of *Ichnocarpus frutescens* (Linn.) R.Br. on paracetamol-induced hepatotoxicity in rats. *Tropical Journal of Pharmaceutical Research*. 2007;6(3):755–765.
2. Zhang A, Sun H, Wang X. Recent advances in natural products from plants for the treatment of liver diseases. *Eur J Med Chem*. 2013;63:570–577.
3. Dey P, Saha MR, Sen A. An overview on drug-induced hepatotoxicity. *Asian J Pharm Clin Res*. 2013;6(4):1–4.
4. Chatterjee TK. Medicinal Plants with Hepatoprotective Properties. Herbal Options Books & Allied (P) Ltd, Calcutta, India. 2000. p.155.
5. Gupta AK, Misra N. Hepatoprotective Activity of Aqueous Ethanolic Extract of Chamomile capitula in Paracetamol Intoxicated Albino Rats. *American Journal of Pharmacology and Toxicology*. 2006;1(1):17–20.
6. Figueirinha A, Paranhas A, Alonso JJP, et al. *Cymbopogon citratus* leaves; Characterization of flavonoids by HPLC-PDA-ESI/MS and an approach to their potential as a source of bioactive polyphenols. *Food Chemistry*. 2008; 110(3): 718–728.
7. Mehraban F, Nasim OT, Fereshteh J. Anti dermatophyte activities of *Eucalytus Camaldulensis* in comparism with Griseofulvin. *Iranian Journal of Pharmacology & Therapeutics*. 2005;4(2):80–83.
8. Chitra R, Sim SM, Ismil R. Effect of *Cymbopogon citratus* and citral on vascular smooth muscle of the isolated thoracic rat aorta. *Evidence-Based Complementary and Alternative Medicine*. 2012;53: 94–175.
9. Naik MI, Fomda BA, Jaykumar E, et al. Antibacterial activity of lemongrass (*Cymbopogon citratus*) oil against some selected pathogenic bacteria. *Asian Pacific Journal of Tropical Medicine*. 2010;3(7):535–538.
10. Shishodia S, Harikumar KB, Dass S, et al. The guggul for chronic diseases ancient medicine, modern targets. *Anticancer Res*. 2008;28(6A):3647–3664.
11. Udeh MU, Agbaji AS, Williams IS, et al. Indica seed oil and essential oils from *Cymbopogon citratus* and *Eucalytus citriodora* leaves. *Ng J Biochem Mol Biol Proc Suppl*. 2001;16: 189–192.
12. Abubakar MC, Ukwuani AN, Shehu RA. Phytochemical screening and antibacterial activity of *Tamarindus indica* pulp extract. *Asian Journal of Biochemistry*. 2008;3(2):134–138.
13. Edeoga HO, Okwu DE, Mbaebie BO. Phytochemical constituents of some Nigeria medicinal plants. *African Journal of Biotechnology*. 2005; 4(7):685–688.
14. Parmar SR, Vashrambhai PH, Kalia K. Hepatoprotective activity of some plants extract against paracetamol induced hepatotoxicity in rats. *J herb med toxicol*. 2010;4:101–106.
15. Trease GE, Evans WL. Pharmacognosy. (16th edn), Bailliere Tindall Ltd; London, USA. 2009.
16. Retimen S, Frankel SA. Colorimetric method for determination of serum glutamic oxaloacetic and glutamic pyruvate transaminases. *Am J Clin Pathol*. 1957;28(1):56–63.
17. King EJ, Armstrong AR. A convenient method for determining of Serum and bile phosphatase activity. *Can Med Assoc J*. 1934;31(4):376–381.
18. Malloy HT, Evelyn KA. The determination of bilirubin with the photometric colorimeter. *J Biol Chem*. 1937;119: 481–490.
19. Gornall AG, Bardwill CJ, David MM. Determination of serum proteins by means of the biuret reaction. *J Biol Chem*. 1949;177: 751–756.
20. Rauber S, Guterres SS, Schapoval EE. LC determination of citral in *Cymbopogon citratus* volatile oil. *J Pharm Biomed Anal*. 2005;37(3):597–601.

21. Tapsell LC, Hemphill I, Cobiack L, et al. Health benefits of herbs and spices: the past, the present, the future. *Med J Aust*. 2006;185(4):4–24.
22. Kumar S, Dwivedi S, Kukreja AK, et al. *Cymbopogon: The Aromatic Grass Monograph*. Lucknow, India. 2000.
23. Vaishwanar I, Kowale CN. Effect of two ayurvedic drugs Shilajeet and Eclinal on changes in liver and serum lipids produced by carbontetrachloride. *Indian J Exp Biol*. 1976;14(1):58–61.
24. Sallie R, Tredger JM, Willaiaam R. Drugs and the liver. *Biopharm Drug Dispos*. 1999;12(4):251–259.
25. Moss DW, Butterworth PJ. *Enzymology and Medicine*, Pitman Medical, London, USA. 1974. p.139.
26. SubramoniamA, Evans DA, Rajasakhran SP. Hepatoprotective activity of *Trichopus zeylanicus* extracts against paracetamol induced damage in rats. *Ind J Expt Biol*. 1998; 36(4):385–389.
27. Koh PH, Mohd AZ, Iqbal M. Antioxidant potential of *Cymbopogon citratus* extract: alleviation of carbon tetrachloride-induced hepatic oxidative stress and toxicity. *Hum Exp Toxicol*. 2012;31(1):81–91.
28. El-Sewry E. The protective effect of lemongrass and its oil on hepatotoxicity in rats caused by CCL4. *New Egypt J Med*. 2011;44(3):58–68.
29. Orhan DD, Aslan M, Aktay G, et al. Evaluation of hepatoprotective effect of *Gentiana oli* Vieri herbs on sub-acute administration and isolation of active principle. *Life Sci*. 2003;72: 2273–2283.
30. Faruq MO, et al. TLC technique in the component characterization and quality determination of Bangladeshi lemongrass oil (*Cymbopogon citratus*) (DC) Stapf). *Bangladesh Journal of Science Industrial Research*. 1994;29(2):27–38.
31. Silva GAB, Bauer L. Volatile constituents of the essential oil of *Cymbopogon citratus* Stapf grown in Zambia. *Flavour and Fragrance Journal*. 1998; 13(1):29–30.
32. Mican KH, Mohammed S. Flavonoid (Myricetin, Quercetin, Kaempferol, Luteolin, and Apigenin) Content of Edible Tropical Plants. *J Agric Food Chem*. 2001;49 (6):3106–3112.