Original Communication

In silico, in-vitro and in vivo screening of biological activities of citral

Shagufta Habib[1], Pawan Gupta[2,3], Sana Shafi Bhat[1], and Jeena Gupta[1]

[1] School of Bioengineering and Biosciences
[2] School of Pharmaceutical Sciences
[3] Department of Research and Development, Lovely Professional University, Phagwara, Punjab, India

Abstract: Citral, one of the main components of lemongrass oil (65–85%), is known to possess various medicinal properties like enhancing skin health and vision-improvement. It also acts as flavoring agent, used in perfumes and skin care products. The objective of this work was to elucidate the biological properties of citral at molecular level using an integrated in silico, in vitro and in vivo approaches. To elucidate this in silico molecular docking studies were performed with in vitro validation by DPPH scavenging activity, MTT assays, enzymatic assays and Chorio Allantoic Membrane (CAM) assay. The in silico analysis demonstrated the potential binding of citral with PPARγ ligand binding domain and vascular endothelial growth factor receptors (VEGFR-1 and VEGFR-2). Citral is already a proven anti-oxidant which is further confirmed by increased DPPH inhibition with increased citral concentration (IC50: 6.9 ± 1.68 μg/ml, $p < 0.05$). The results demonstrated that citral protect yeast cells from cytotoxic effects of hydrogen peroxide and also increase the activities of antioxidant enzymes like GST, SOD and LPO. It was also demonstrated to be cytotoxic to cancerous HeLa cells (IC50: 3.9 ± 0.38 μM, $p < 0.01$) and was found anti-angiogenic by CAM assay. This study highlights many important pharmaceutical properties of citral which can be explored further to increase its industrial applications.

Keywords: Citral, PPARγ agonist, similarity searching, molecular docking, vascular endothelial growth factor receptors

Abbreviations

PPAR	Peroxisome proliferator-activated receptors
VEGFR	Vascular Endothelial Growth Factor Receptors
DPPH	2,2-diphenyl-1-picrylhydrazyl
MTT	3-(4,5-Dimethyl-2-thiazolyl)-2,5-diphenyl-2H-tetrazolium bromide
GST	Glutathione-S-Transferase
SOD	Superoxide Dismutase
CAM	Chorio Allantoic Membrane
PDB	Protein Data Bank
ADT	Auto Dock Tools
DMSO	Dimethyl sulfoxide
CDNB	1-chloro-2,4-dinitrobenzene
LPO	Lipid Peroxidation
BSA	Bovine serum albumin
MDA	Malondialdehyde

Introduction

Natural products isolated from plants are important for both pharmacists and nutritionists to develop new drugs and food supplements respectively. They have been used since thousands of years for the treatment and prevention of diseases [1]. Lemon grass oil is having a characteristic sweet lemony smell, dark yellow color and is a valuable raw material for the manufacturing of citrus-type soaps, cosmetics, toiletries and insect deterrents [2]. Citral (3,7-dimethyl-2,6-octadienal) is its main component, which is a mixture of two open-chain monoterpenoids: Geraniol and Neral and is enriched in volatile oils of several other plants like lemon, Litsea, clove and basil. It's been used extensively as flavouring agent and in fragrances for its rich lemon aroma [3]. Citral is known to stimulate nervous system and possess deodorizing, carminative and diuretic properties [4]. It is a bioactive molecule and known to exert many pharmacological effects like anti-inflammatory [5], antibacterial [6, 7], antifungal [8] and antitumor activity [9]. Citral had also been demonstrated to reduce inflammation markers like plasma cytokines and prostaglandin E2 and showed anti-pyretic effects in rats administered systemically with lipopolysaccharides [10]. Because of these effects of citral, it is widely used as food additive for health promotion.

Citral is also known to be an activator of peroxisome proliferator-activated receptor gamma (PPARγ). PPARγ

emerges as a valuable drug target against metabolic diseases as it works as nuclear transcription factor and regulates genes expression for glucose metabolism, adipogenesis and lipid homeostasis [11]. The synthetic PPAR agonists such as thiazolidinediones and rosiglitazone entered into the market globally around 1999–2000. However, their notable side effects to focus were abnormal liver function, fluid retention and cardiac failure [12]. Therefore researcher are finding an alternative way and moving to find partial PPARγ agonists from natural products which possess therapeutic effects against type 2 diabetes. Citral had been shown to activate PPARγ and suppresses the expression of cyclooxygenase-2 [13]. It had also been demonstrated to inhibit the acute lung injury induced by lipopolysaccharides by activating PPARγ [14]. However limited literature is present to explore the biological activities of citral and its interactions with the target proteins.

The present study aimed to explore the various biological activities of citral using an integrated in silico, in vitro and in vivo approach. The potential binding of citral with protein targets like PPARγ and Vascular Endothelial Growth Factor Receptors (VEGFRs) was explored by molecular docking studies. VEGFRs are receptor tyrosine kinases which bind with VEGF and initiate signaling cascades to stimulate angiogenesis. The study demonstrated the antiangiogenic property of citral using in vivo Chick Chorioallantoic Membrane (CAM) Angiogenesis Assays. Furthermore, citral was also found to inhibit the proliferation of cancerous HeLa cells. To best of the knowledge this is the first report which explores the in silico binding of citral with PPARγ and VEGFRs and also demonstrated its anti-angiogenic and anti-cancer potential.

Material and methods

Materials

In this study, all the chemicals were provided by Hi-Media Co. unless otherwise mentioned. Purified citral was purchased from Sigma Aldrich (CAS No. 5392-40-5).

Molecular docking

Molecular docking is a process to predict the binding conformations of the query structure into the active site of the protein. AutoDockVina, version 1.5.6 by Scripps Research Institute [15] was used for the docking studies of citral into the active site of PDB protein.

For docking, a protein structure was obtained from PDB and 3D structure of citral was obtained from chemical database (CHEMBL, Pubchem, etc). The bound similar molecule was extracted from the PDB file. All the structures were automatically prepared before docking in AutoDock Vina. AutoDock tools 1.5.6 (ADT) were used to prepare the molecule (deleting all water molecules, adding polar hydrogen's and loading Kollman United Atoms charges) and also to perform docking calculations. A grid box with spacing 0.375 and dimensions 50 × 50 × 50 points was constructed around the binding site, based on the location of the co-crystallized ligand. All bond rotations and torsions for the ligand were automatically set in the ADT. In AutoDock Vina, first bound molecule in protein was docked into the active site of protein to check the docking program if it is generating the same binding modes in PDB or not. Using the same docking parameters, docking with citral was then performed. The interpretations of the docking results were done to analyze the predicted binding interactions.

DPPH Free Radical Scavenging Activity

Free radical scavenging activity was determined by observing DPPH (1, 1 diphenyl-2-picrylhydrazyl) inhibition following the method described by [16]. The concentrations of citral used were 0.5 μg/ml, 1 μg/ml, 1.5 μg/ml, 2 μg/ml and 2.5 μg/ml. into a sample mixture having a final DPPH radical concentration of 0.1 mM. The mixture was shaken vigorously (2,500 rpm) for 1 min then left to stand for 60 min in the dark. Scavenging capacity was measured spectrophotometrically at 517 nm. Ascorbic acid (0.5 μg/ml) was used as a positive control. Inhibition (%) was plotted against the sample concentration in the reaction system. The percentage inhibition of the DPPH radical calculated according to the following formula:

$$\%\,\text{Inhibition} = [(\text{A control} - \text{A sample})/\text{A control}] \times 100$$

where A is absorbance.

Dose Response

Yeast Potato dextrose broth containing equal amount of yeast cells (Saccharomyces cerevisiae) with OD 0.1 were incubated with different concentrations of citral and H_2O_2. The concentrations used were 10 μg/ml, 20 μg/ml, 30 μg/ml, 40 μg/ml and 50 μg/ml for citral and 3 mM, 209 6 mM, 9 mM and 12 mM for H_2O_2. Test tubes were placed in shaking incubator at 30 C for 24 hours and absorbance was measured at 600 nm.

Cell Cytotoxicity Test

MTT assay was used to visualize the cell cytotoxicity and viability as described by Garlier and Thomasett, 1984 [17]. An inoculum of overnight culture of Saccharomyces

cerevisiae was transferred to fresh Yeast Potato dextrose broth and incubated with shaking at 30 °C until late exponential phase was reached. Aliquots (1.0 ml), containing approximately 1×10^8 cells/ml, were pipetted into Eppendorf tubes and H_2O_2 (12 mM), citral 0.5 mg/ml were added and tubes were incubated at 22 °C for 90 minutes. Tubes were centrifuged and cells washed with autoclaved water. Cells were suspended in 120 µL water and 80 µL of 50 mg/mL MTT was added. Incubated for 3 hours in shaking incubator, centrifuged for 10 minutes and equal amount of DMSO (dimethyl sulfoxide) was added. Cells were centrifuged and absorbance in the supernatant was measured at 570 nm. The absorbance at 570 nm was positively correlated to the number of viable cells, so the cell viability was represented as the percentage cell survival between treated and untreated cells.

MTT assay in Hela cells

Hela cells purchased from National Centre For Cell Science, Pune, India, were seeded at a density of 1×10^4 cells/mL in 96-well culture plates at 37 °C with 5% (v/v) CO_2 for 24 h. After incubation, cells were incubated with different concentrations of citral (3, 6, 12 and 24 µg/mL). Control cells received only culture medium. Then, 100 µl of MTT solution (5 mg/mL) was added to each well and the plates were incubated at 37 °C for an additional 4 h. After dissolving the formazan crystals in DMSO, absorbance was measured in each well at 570 nm by ELISA Plate Reader. Cell viability (%) was calculated for all groups compared to control sample. All experimental samples were performed in triplicate.

Antioxidant Enzyme activity

Estimation of Superoxide dismutase (SOD) activity

The Superoxide dismutase activity was measured by following method given by Beauchamp and Fedovich [18]. Briefly to 50 µl of cell lysate, 1.3 ml of 50 mM sodium carbonate buffer in 0.1 mM EDTA (pH = 10.8), 0.5 ml of 96 µM NBT and 0.1 ml of 0.6% Triton X-100 were added. The addition of 0.1 ml of 20 mM Hydroxylamine hydrochloride initiate the reaction and the absorbance was measured at 560 nm at 30 seconds interval for 5 minutes using spectrophotometer. The unit SOD activity was calculated as an amount of enzyme required for 50% inhibition of NBT reduction. The specific activity was then calculated as units per mg protein.

Estimation of Glutathione-S-Transferase (GST)

The method by Habig et al. [19] was used to estimate GST activity. The reaction mixture contained 1000 µl of phosphate buffer (pH 6.5; 0.1 M), 300 µl of reduced glutathione (1 mM), 50 µl of 1 mM 1-chloro-2,4-dinitrobenzene (CDNB) and 100 µl of cell lysate in 2000 µl of total volume. The increase in absorbance due to the formation of glutathione conjugate was recorded in UV-VIS spectrophotometer at 340 nm for 5 minutes at interval of 60 seconds. The enzyme activity was calculated as nM CDNB conjugate formed per min/mg protein and the molar extinction coefficient used here was $9.6 \times 10^{-3}\ M^{-1}\ cm^{-1}$.

Estimation of lipid Peroxidation (LPO)

The protocol by Iqbal et al. [20] was used to measure LPO level. Briefly, the reaction mixture comprised of 1000 µl of Tris-HCl buffer (pH 7.1; 150 mM), 100 µl of cell lysate, 100 µl of ferrous sulfate (1 mM) and 100 µl of ascorbic acid (1.5 mM) in a total 1000 µl volume. The reaction mixture was incubated at 37 °C for an hour. The reaction was stopped by adding 1000 µl of 10% trichloroacetic acid. After the addition of 1000 µl of 0.375% thiobarbituric acid, tubes were plugged and kept for 15 min in boiling water and then placed on ice bath and centrifuged at 3000×g for 10 min. The quantity of TBARS (lipid peroxidation) was calculated by taking the absorbance of supernatant contrary to a reagent blank at 535 nm. The results were articulated as nM MDA formed /min/mg tissue at 37 °C using molar extinction coefficient of $1.56 \times 10^{-5}\ M^{-1}\ cm^{-1}$.

CAM assay for Anti-Angiogenic activity

CAM assay was performed on fertilized hen egg. On the 5th day of incubation, eggs were windowed aseptically utilizing dissecting devices [21]. A small window (roughly 2 cm in diameter) was made by removing the outer shell and internal membrane from the air-space site. Around the same time, 4 – 5 ml of egg albumin was suctioned with a sterile syringe to enable the embryo to develop. Filter discs were utilized to insert the test material to a particular area of the CAM. The discs were pre-absorbed in test compound (citral) or vehicle (DMSO) and were embedded on the CAM. For the administration of citral, nitrocellulose membrane was prepared with methyl cellulose, heparin and citral (10 µl). The window was closed utilizing micropore tape and the eggs were placed into the incubator. 3 days after implantation, the window of the eggs was opened up followed by counting of the number of blood vessels in the zone of the disc. Inhibition of angiogenesis by anti-angiogenic compounds was observed as the absence of new blood vessels formation and very often by vanishing of pre-existing blood vessels.

Protein Estimation

Protein concentration was determined following the standard method given by Lowry et al., 1951 [22] using bovine serum albumin (BSA) for making standard curve.

Statistical analysis

Experimental values are expressed as mean ± SEM. Comparison of mean values between various groups was performed by one way-analysis of variance (one way-ANOVA) followed by Turkey's test using GraphPad Prism software. P-value < 0.05 was considered to be significant.

Results

Molecular docking of citral with PPARγ

We have performed molecular docking of citral with human PPARγ ligand binding domain. For this first water and B chain from protein structure were removed, missing atoms were repaired, added polar hydrogens, Kollman charges and merged the non-polar ones. It was visualized that the citral was having similar binding poses as unique ligand 2PQ (2-[(1-{3-[4-(biphenyl-4-ylcarbonyl)-2-propyl-phenoxy] propyl}-1,2,3,4-tetrahydroquinolin-5-yl)oxy]-2-methylpropanoic acid) with PPARγ ligand binding domain (Figure1). The surface view of human PPARγ ligand binding domain (Figure 1B) demonstrated that citral occupies a cavity by forming one hydrogen bond with Ser-342 (distance- 0.34Å). The other interacting amino acids include Lys461 and Phe 247 and the estimated free energy of binding was –5.4 Kcal/mol (Table 1).

Docking studies of citral against VEGFR

For VEGFR-1 and VEGFR-2 (PDB: 5ABD and 5OYJ), first co-crystallized ligands (SO4 and Acetate respectively) were extracted. Then docking of citral was performed into the active site of these proteins by using same parameters as their respective co-crystallized ligands. Citral was found to possess surface binding with VEGFR-1, interacting with amino acids His 223 and Arg 224, forming no hydrogen bond. The estimated free energy of binding was –3.2 Kcal/mol (Figure1C and 1D). Furthermore, citral was found to bind into the center of the binding pocket of VEGFR-2 interacting with multiple amino acids like His546, Asn468, Ile429, Val 432 and Ser544 (Figures 1E and 1F). However, there was no hydrogen bond formation and the estimated free energy of binding was −4.2 Kcal/mol.

Antioxidant activity of citral

The results showed that citral exhibit antioxidant activity as there was an increase in % inhibition of free radical DPPH (8.26 ± 0.101, 10.87 ± 0.077, 17.39 ± 0.096, 22.83 ± 0.12 and 32.17 ± 0.22) with increasing citral concentration (0.5 μg/ml, 1 μg/ml, 1.5 μg/ml, 2 μg/ml and 2.5 μg/ml; Figure 2). The IC50 value was determined to be 6.9 ± 1.68 μg/ml which highlights the strong antioxidant activity of citral.

Cytotoxic effect of citral and H_2O_2 on yeast cells at different doses

Yeast is model eukaryotic organism for elucidation of different biological activities. To evaluate the cytotoxic effects of citral and H_2O_2 in yeast cells, cells were incubated with different concentrations of citral (10 μg/ml, 20 μg/ml, 30 μg/ml, 40 μg/ml and 50 μg/ml) and H_2O_2 (3 mM, 6 mM, 9 mM and 12 mM) respectively at 30 °C for 24 hours. The cell density was measured by taking OD at 600 nm. No cytotoxicity was observed by increasing citral concentration, however, increasing H_2O_2 concentration progressively decreased cell density. In another setup, the cells were pre-incubated with citral (0.5 mg/ml) and 12 mM of H_2O_2 and MTT assay was performed to check percentage cell viability. It was observed that citral rescued the cells from the cytotoxic effects of H_2O_2 (Figures 3A, 3B and 3C).

Effect of citral treatment on Anti-oxidant enzymes

We have further evaluated the effect of citral treatment on activity of three antioxidant enzymes: GST, SOD and LPO. Increased GST activity, SOD activity and LPO were observed in citral treated cells as compared to control cells. The measured rate of CDNB- glutathione conjugate formation was 1.2139 ± 0.14 μmoles/mg/minute (for GST activity), as compared control cells with 0.136 ± 0.009 μmoles/mg/minute. LPO was also increased in citral treated cells with 2.9 ± 0.53 μM MDA/min/mg protein as compared to control cells 0.58 ± 0.012 MDA/min/mg protein. Citral treatment (50 mg/ml) also markedly increased Superoxide dismutase activity in cells with 21.5 ± 0.3 Units SOD activity/mg protein as compared to control cells 8.29 ± 0 Units SOD activity/mg protein (Figure 4).

Cytotoxic effects of citral on Hela cells

To check the effect of citral on in vitro viability of cervical epithelioid carcinoma (Hela) cells, MTT assay was performed with different concentrations of citral. As shown in Figure 5, treatment with 30, 60, 120 and 240 μM of citral significantly decreased the cell viability (IC50: 3.9 ± 0.38 μM). Thus citral is cytotoxic to the cervical epithelioid cancerous cell and thus possesses anti-cancer activity.

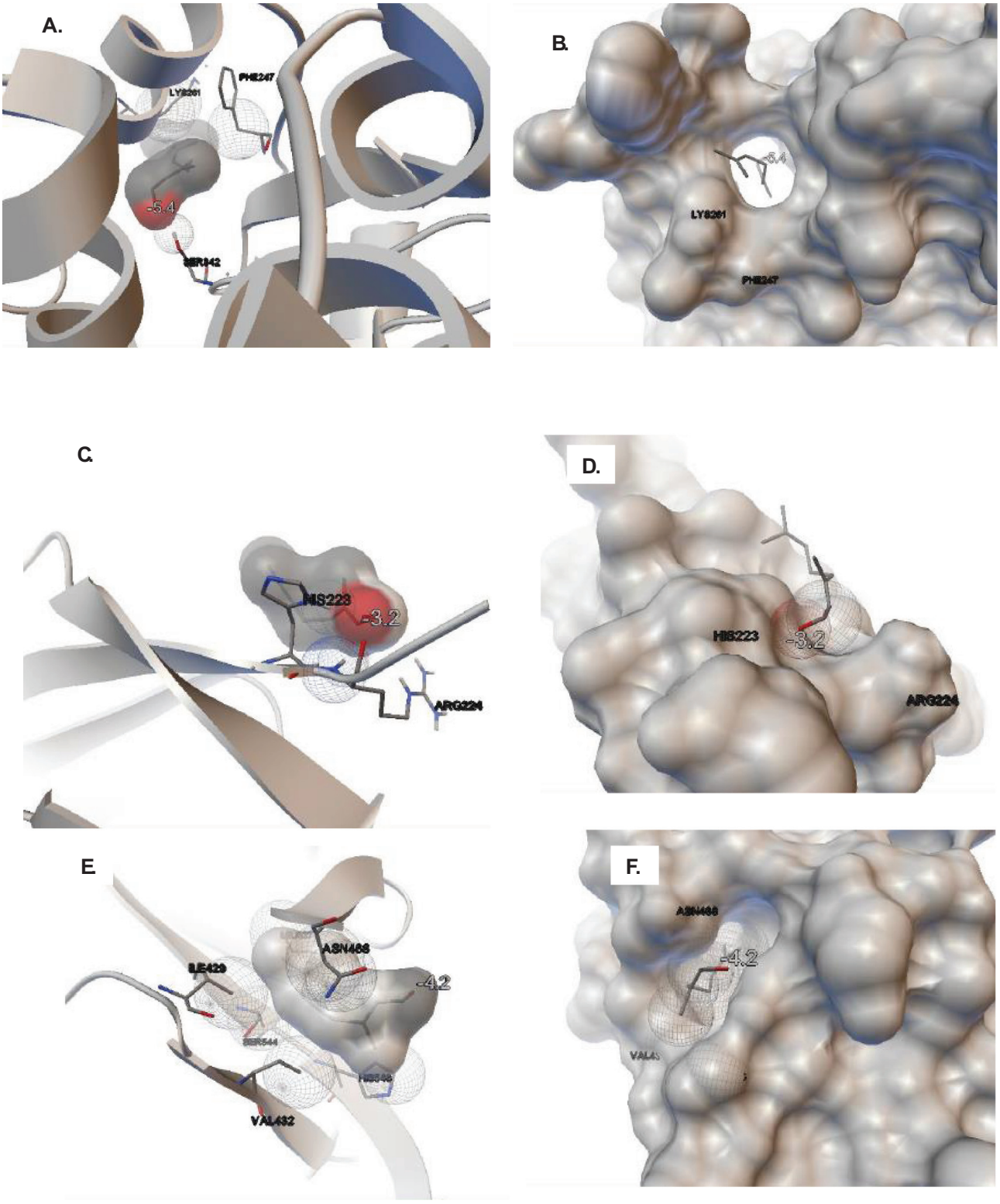

Figure 1. Molecular docking of citral. A and B with Protein 3GBK (PPARγ ligand binding domain). A: Interacting amino acids and B: Surface interaction of PPARγ ligand binding domain with citral. C and D with crystal structure of 5ABD protein (VEGFR-1). C: Interacting amino acids and D: Surface interaction of VEGFR-1 with citral. E and F with crystal structure of 5OYJ protein (VEGFR-2). E: Interacting amino acids and F: Surface interaction of VEGFR-2 with citral.

Table 1. Estimated free energy of binding of citral with different receptors.

Serial no.	Ligand name	Protein name	Est. Free binding energy	Interacting amino acids
1	Citral	3GBK (PPARγ ligand binding domain)	−5.4 kcal/mol	Ser342 Lys 261 Phe 247
2	Citral	5ABD (VEGFR1)	−3.2 kcal/mol	Arg 224 His 223
3	Citral	5OYJ (VEGFR2)	−4.2 kcal/mol	His 546 Asn 468 Ile 429 Val 432 Ser 544

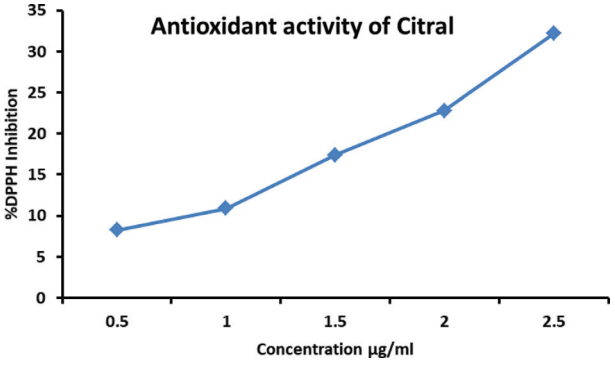

Figure 2. Antioxidant activity of citral. Percentage DPPH inhibition was measured using increasing concentrations of citral by DPPH assay. Similar results were obtained in the three independent set of experiments. All the values were represented as mean ± S.E.M. (n = 3), ***p < 0.001, **p < 0.01 and *p < 0.05, vs control.

Anti-angiogenic activity of citral in CAM assay

The number of blood vessels and the micro vessels formed after the infusion of the citral is shown in the Figure 6. It was seen that the blood vessel development was reduced significantly ($p < 0.001$) in the citral treated group as examined in terms of number of blood vessels and branching points in comparison with the control group (respectively). The number of branch points seen in the vehicle control group was 21 ± 3 whereas only 7 ± 3 branches were seen in citral treated group.

Discussion

The growing realization on the potential of natural products to promote health and to be used as drugs against various diseases proves a boon for pharmaceuticals industries. The oil of an aromatic plant; Lemongrass (family: Gramineae) is having various uses in food and perfumery industry [23]. Citral is its major constituent present at a

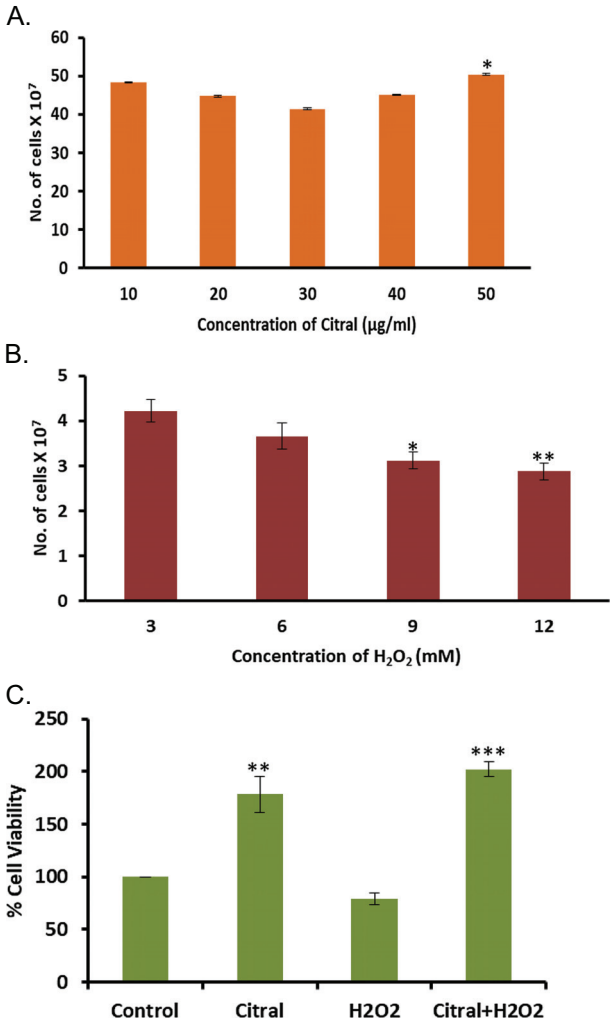

Figure 3. Cytotoxic effects of citral. Yeast cells were cultured in the presence of different concentrations of citral till exponential phase. After incubation the O.D. was taken at 600 nm and the effect of citral on yeast cells were measured. Bar graph A. Dose response of citral on yeast cells, B. Dose response of H_2O_2 with citral treatment at 500 µg/ml concentration and C. Percentage cell viability calculated by MTT assay after treatment of the yeast cells with citral 500 µg/ml. Each value represents the mean ± sd of three experiments. (n = 3). All the results are represented as Mean ± SEM (n = 3). ***p < 0.001, **p < 0.01 and *p < 0.05, vs control.

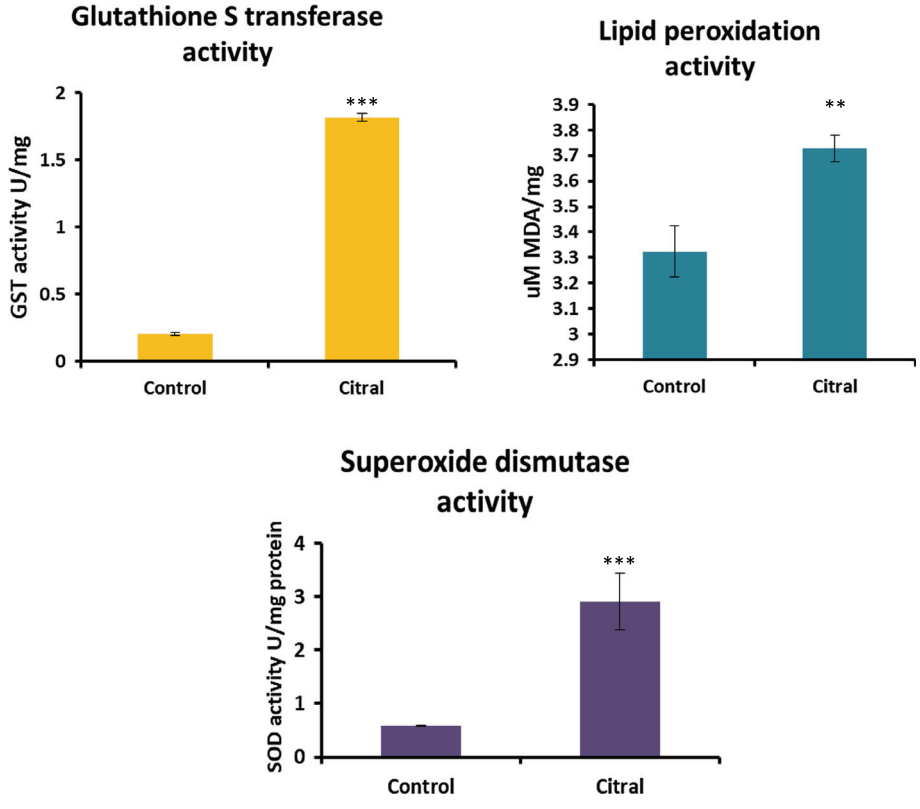

Figure 4. Effect of citral on activity of anti-oxidant enzymes in yeast cells. Yeast cells were cultured in the presence citral (50 μg/ml) till exponential phase. Cells were homogenized and GST, SOD and LPO activity was measured. Similar results were obtained in the three independent set of experiments. All the values were represented as mean ± S.E.M. (n = 3), ***p < 0.001, **p < 0.01 and *p < 0.05, vs control.

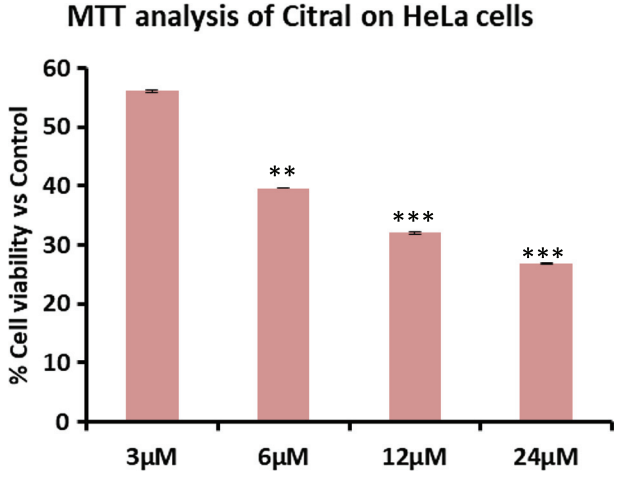

Figure 5. Anti-cancerous activity of HeLa cells. Hela cells were seeded at a density of 1×10^4 cells/mL in 96-well culture plates at 37 °C with 5% (v/v) CO2 for one day. After 24 h of incubation, cells were incubated with citral at the indicated concentrations for 2 h and percentage cell viability calculated by MTT assay.

concentration ranging between 75% – 85% and is majorly responsible for its strong lemony odor. The quality of lemongrass oil is determined by citral content where higher citral concentration correlates to higher purity [24]. Citral also emerges as a potential drug candidate and was also found to possess diuretic, carminative and stimulative effects on central-nervous-system [25]. However, in depth evaluation of its biological activity and toxicity is required to enhance their safety and efficacy.

Molecular docking studies shows potential binding of citral with PPARγ ligand binding domain by forming one hydrogen bond with Ser-342. Previous reports have highlighted that most of the partial PPARγ agoinsts form hydrogen bond with Ser-342 [26]. This may project partial PPARγ agonistic activity of citral. PPARγ agonist plays a crucial role in regulating anti-inflammatory responses. Previous reports have demonstrated the anti-inflammatory properties of lemongrass oil and the topical application of lemongrass essential oil was found to inhibit inflammatory response in mice skin [27]. It reduced benzo-α-pyrene induced DNA damage and oxidative stress in human embryonic lung fibroblasts [28]. Lemongrass oil has also been accounted to inhibit the expression of inflammatory cytokines, such as IL-1β and IL-6, in peritoneal macrophage [29].

Citral was found to possess significant antioxidant activity with IC50 of 6.9 ± 1.68 μg/ml by DPPH assay. Citral had been previously reported to suppress the expression of the inducible nitric oxide synthase (iNOS) gene by LPS and to suppress the DNA-binding activity of the NF-κB site in the iNOS gene [30]. The effect of citral treatment on activity

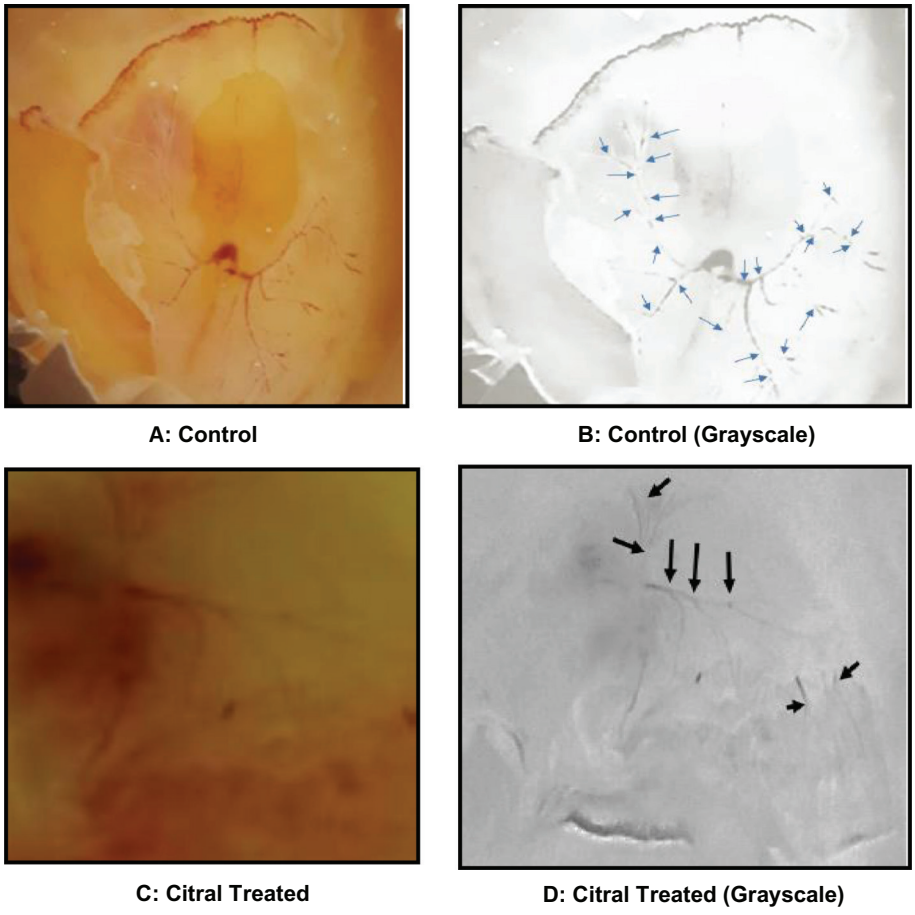

Figure 6. Anti-angiogenic activity of ctral by CAM assay. Arrows represent branching points; where A: Control, B: Control (Grayscale), C: Citral treated, D: Citral treated (Grayscale).

of three antioxidant enzymes: GST, SOD and LPO were further evaluated. Increased GST activity, SOD activity and LPO were observed in citral treated cells. Increased activity of these anti-oxidant enzymes is an indicator that citral may increase the potential of cells to rescue from oxidative stress [31]. Previously, citral when given in combination with aspirin; protects IEC-6 cells from aspirin-induced cell death and also modulate the activities of anti-oxidant enzymes like glutathione (GSH) and SOD [32].

The effect of citral on the in vitro viability of cervical epithelioid carcinoma (Hela) cells was evaluated by performing MTT assay with different concentrations of citral. It was observed that citral was cytotoxic to the cervical epithelioid cancerous cell and thus possesses anti-cancer activity. Furthermore the anti-angiogenic activity of citral was also observed by CAM assay which may be the result of its interaction with VEGFRs (VEGFR-1 and VEGFR-2, shown by molecular docking studies, Figures 1C, 1D, 1E and 1F). Lemongrass oil had been previously reported to have promising anticancer activity and to cause loss of tumor cell viability [33].

Citral is a major component of many food products like lime, lemon and oranges (concentration varies between 1–9% w/w). The concentration of citral is 1.5 to 2 times higher in green lemon in comparison to yellow lemons which is also responsible for its antifungal activity. The recent trend highlighted that improving the selection criteria for high oil yielding germplasms of lemongrass helps in improving its applicability [34, 35]. However long term storage decreases the citral concentration in lemon fruit with increased incidence of decay and declined antifungal potential [36]. Citral is also an unstable molecule and is prone to oxidation on exposure to air. Therefore different strategies like reducing the storage temperature, alterations in environmental stresses pH and oxygen partial pressure have been employed to limit the degradation of citral oils but these approaches are less practical for maintaining the integrity of food products. Also, there are reports which highlighted that citral cause considerable liver injury when given at higher dose (2000 mg/kg). It was found to nearly double SGPT and SGOT levels in rats as compared to control [37]. However, lower dose of citral (125–500 mg/kg) was found protective against liver toxicity induced by acetaminophen [38]. This highlights that higher dose of citral must be avoided and citral containing products require proper storage conditions.

Conclusion

The health community increasingly recognizes the critical role of nutrition in human well-being. These priorities are recognized by striking increase of investment in nutritional products in recent times. Pharmaceutical industries are always looking towards molecular entities with health benefits to make new formulations. Pure phytochemicals are always the first target of pharmaceutical industries for new drug development because of their abundance, easy availability and less cost. The study highlights that citral, a phytoactive component abundantly present in lemongrass shows potential binding with PPARγ, VEGFR-1 and VEGFR-2. Furthermore, it was found to possess many important pharmaceutical properties like anti-oxidant anti-angiogenic and anti-cancer activities and was also found to regulate the activity of anti-oxidant enzymes. These biological properties of citral can be explored further to provide other health benefits. More insight understanding is required to check the bioavailability, dosage and tissue distribution of citral either alone or in combination with other dietary components to obtain the maximum beneficial effects.

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Conflict of interest
The authors declare that there are no conflicts of interest.

Authors contribution
Shagufta Habib had performed in silico studies and all experiments in the laboratory. Dr. Pawan have planned and guided the student to execute the in-silico work. Sana Shafi had done data analysis and formatting of the manuscript. Dr. Jeena Gupta have supervised the whole study, edited and formatted the manuscript.

Dr. Jeena Gupta
Assistant Professor
Department of Biochemistry
School of Bioengineering and Biosciences
Lovely Professional University (LPU)
Jalandhar – Delhi G.T. Road, Phagwara
Punjab (India) – 144411
jeena_gupta@yahoo.co.in

