Alanine Aminotransferase (ALT), Acid Phosphatase (ACP), Alkaline Phosphatase (ALP), Lactate Dehydrogenase (LDH), Gamma Glutammyl Transpeptidase (GTT), Alpha Feto-Protein (AFP) and 5'Nucleotidase levels.

2.3 Supernatant Preparation

The liver samples were harvested from the experimental animals and weighted and perfused immediately with ice-cold saline (0.85%, w/v NaCl), and homogenized with chilled phosphate buffer (0.1M, pH 7.4) containing KCl (1.17%, w/v). The homogenate was then centrifuged (800 g, 5 min, 4 °C) for removing the cell debris. The supernatant was collected and centrifuged at 10,000 g for 20 min at 4° C. Then the resulting supernatant is then assayed for CAT, SOD, and GPx activities.

2.4 Determination of the Extract Effect on Lipid Peroxidation in Liver

Lipid peroxidation was estimated by Thiobarbituric Acid (TBA) reaction with Malondialdehyde (MDA) as reported earlier[22]. Lipid peroxidation was expressed as nanomoles of MDA per milligram of protein.

2.5 Determination of the Extract Effect on Antioxidant Enzyme Activities in the Liver

2.5.1 Superoxide Dismutase Activity

The SOD activity was measured according to the method used by Marklund and Marklund[23]. The enzyme activity was expressed as units/mg protein.

2.5.2 Catalase Activity

The protein content of the supernatant was determined using the method with copper sulphate as reported earlier[24]. The enzyme activity was calculated as nano mole of H_2O_2 consumed/min/mg protein.

2.5.3 Glutathione Peroxidase Activity

To estimate the GPx activity, the reaction mixture consisted of 1.65 mL phosphate buffer (0.1 M, pH 7.4), 0.1 mL EDTA (0.5 mM), 0.05 mL oxidized glutathionee (1 mM), 0.1 mL NADPH (0.1 mM), and 0.1 mL supernatant in a total volume of 2 mL. The disappearance of NADPH at 340 nm was recorded at 25 °C. The enzyme activity was calculated as nano mol of NADPH oxidized/min/mg protein using molar extinction coefficient.

3. Results

The activities of enzymatic antioxidants (SOD, CAT and GPx) level in the liver hemogenate of the experimental animals in each group are shown in Table 1.

The activities of SOD, CAT and GPx level were significantly decreased in hemogenate of tumor bearing animals (Group II) as compared to control animals. Geraniol administrated rats showed significant increase in the enzymatic antioxidants levels (Group III and Group IV).

The activities of enzymatic antioxidants (SOD, CAT and GPx) level in liver of the experimental animals in each group are shown in Table 2.

The activities of SOD, CAT and GPx level were significantly decreased in liver tissue of tumor bearing animals (Group II) as compared to control animals. Geraniol administrated rats showed significant increase in the enzymatic antioxidants levels (Group III and Group IV).

The activities of non-enzymatic antioxidants (GSH, Vit C and Vit E) level in haemolysate of the experimental animals in each group are shown in Table 3.

Table 1. Effect of geraniol on the enzymatic antioxidants in liver hemogenate of control and experimental animals

Parameters	Superoxide dismutase (SOD)	Catalase (CAT)	Glutathione peroxidase (GPx)
Group I (Control)	3.72 ± 0.08	65.92 ± 4.23	6.52 ± 0.25
Group II (DEN)	2.34 ± 0.33^{a***}	42.44 ± 2.42^{a***}	3.81 ± 0.32^{a***}
Group III (DEN + Geraniol)	3.38 ± 0.03^{b***}	57.25 ± 4.49^{b***}	4.40 ± 0.33^{b**}
Group IV (Geraniol)	3.59 ± 0.19^{aNS}	64.68 ± 2.76^{aNS}	6.42 ± 0.28^{aNS}

Values represent mean ± SD for 6 rats in each group.

Units: SOD – units /mg protein

CAT – μ moles of H_2O_2 utilized/min/mg protein.

GPx – μ moles of GSH oxidised /min/mg protein.

$^{***}p<0.001$; $^{**}p<0.01$; $^{*}p<0.05$

^{NS} Non-significant.^{a} when compared with group I. ^{b} when compared with group II.

The activities of GSH, Vit C and Vit E level were significantly decreased in haemolysate of tumor bearing animals (Group II) as compared to control animals. Geraniol administrated rats showed significant increase in the non-enzymatic antioxidants levels (Group III and Group IV).

The activities of non-enzymatic antioxidants (GSH, Vit C and Vit E) level in liver of the experimental animals in each group are shown in Table 4.

The activities of SOD, CAT and GPx level significantly decreased in liver tissue of tumor bearing animals (Group II) as compared to control group. Geraniol administrated rats showed significant increase in the enzymatic antioxidants levels (Group III and Group IV). Geraniol administrated rats showed significant increase in the enzymatic antioxidants levels (Group III and Group IV).

Table 2. Effect of geraniol on the enzymatic antioxidants in liver of control and experimental animals

Parameters	Superoxide dismutase (SOD)	Catalase (CAT)	Glutathione peroxidase (GPx)
Group I (Control)	8.14 ± 0.47	60.88 ± 3.39	5.40 ± 0.30
Group II (DEN)	4.41 ± 0.34^{a***}	43.05 ± 1.56^{a***}	2.77 ± 0.42^{a***}
Group III (DEN + Geraniol)	7.30 ± 0.42^{b***}	54.23 ± 0.61^{b***}	4.43 ± 0.36^{b***}
Group IV (Geraniol)	7.87 ± 0.19^{aNS}	60.25 ± 1.15^{aNS}	5.16 ± 0.11^{aNS}

Values represent mean ± SD for rats in each group.
Units: SOD – units /mg protein
CAT – µ moles of H_2O_2 utilized/min/mg protein.
GPx – µ moles of GSH oxidised /min/mg protein.
$^{***}p<0.001$; ** $p<0.01$; * $p<0.05$
^{NS} Non-significant. ^{a} when compared with group I. ^{b} when compared with group II.

Table 3. Effect of geraniol on the non-enzymatic antioxidants in haemolysate of control and experimental animals

Parameters	Glutathione reduced (GSH)	Vitamin C	Vitamin E
Group I (Control)	3.19 ± 0.29	1.90 ± 0.06	1.41 ± 0.11
Group II (DEN)	1.29 ± 0.04^{a***}	0.84 ± 0.03^{a***}	0.71 ± 0.04^{a***}
Group III (DEN+Geraniol)	2.76 ± 0.27^{b***}	1.79 ± 0.12^{b***}	1.26 ± 0.15^{b***}
Group IV (Geraniol)	2.85 ± 0.30^{aNS}	1.86 ± 0.04^{aNS}	1.24 ± 0.14^{aNS}

Values represent mean ± SD for 6 rats in each group.
Units: GSH, vitamin C, vitamin E- µg/mg protein.
$^{***}p<0.001$; ** $p<0.01$; * $p<0.05$
^{NS} Non-significant. ^{a} when compared with group I. ^{b} when compared with group II.

Table 4. Effect of geraniol on the non-enzymatic antioxidants in liver of control and experimental animals

Parameters	Glutathione reduced (GSH)	Vitamin C	Vitamin E
Group I (Control)	4.46 ± 0.35	2.81 ± 0.26	1.83 ± 0.05
Group II (DEN)	2.33 ± 0.16^{a***}	1.33 ± 0.08^{a***}	0.84 ± 0.02^{a***}
Group III (DEN+Geraniol)	3.83 ± 0.13^{b***}	2.45 ± 0.16^{b***}	1.72 ± 0.18^{b***}
Group IV (Geraniol)	4.20 ± 0.16^{aNS}	2.60 ± 0.30^{aNS}	1.80 ± 0.06^{aNS}

Values represent mean ± SD for 6 rats in each group.
Units: GSH, Vitamin C, Vitamin E- µg/mg protein
$^{***}p<0.001$; ** $p<0.01$; * $p<0.05$
^{NS} Non-significant.^{a} when compared with group I. ^{b} when compared with group II.

4. Discussion

In the present study we demonstrate that the monoterpene geraniol has antioxidant activity. Geraniol, an acyclic monoterpene alcohol found in lemongrass and aromatic herb oils, has been shown to exert in vitro and in vivo antitumor activity against murine leukemia, hepatoma, and melanoma cells[25–27].

M. S. Seema Farhath et al.[28] studied the Antioxidant activity of Geraniol, Geranial acetate, Gingerol and Eugenol was employed by two complementary test systems, namely, 2,2-diphenyl-1-picrylhydrazyl (DPPH) free-radical scavenging and Super Oxide Dimutase (SOD) activity. Antioxidant activity of Gingerol was found to be higher than those of the others in DPPH assay.

Geraniol, a colourless liquid, is an acyclic terpene alcohol with a flowery rose-like odour. It is found widely as a chief constituent in essential oils including orange flower oil, lemon grass oil and lavender oil. Experimental studies demonstrated several pharmacological activities including antioxidant and anticancer potential of geraniol[29].

Geraniol exerted anti-tumor activity against various cancer cells both in vitro and in vivo[30–32]. It has also been reported that geraniol exhibited potent insecticidal, antimicrobial and anti-inflammatory effects[33,34] reported that dietary geraniol suppressed hepatic HMG CoA reductase activity and lowered the levels of serum cholesterol in experimental animals.

Artega et al.[35] performed extend study focused on the antioxidant activity of low-molecular antioxidants present in the spice and some pharmaceuticals (gallic acid, sesamol, eugenol, thymol, carvacrol, vanillin, salicylaldehyde, limonene, geraniol, 4-hexylresorcinol).

Hanaa H et al.[36] studied the essential oil Dracocephahan moldavica L by TLC, GC-MS, HPLC and NMR and its constituents were gerneyl acetate, geraniol, nerol, neryl acetate, neral and linolool. This oil showed antimicrobial and antioxidant activity.

S. Manoharan et al.[37] investigated the chemopreventive potential of geraniol, an acyclic monoterpene alcohol, by monitoring the tumor incidence and analyzing the status of phase II detoxification agents, lipid peroxidation by products and antioxidants in 7,12-dimethylbenz(a) anthracene (DMBA) induced mouse skin carcinogenesis and found that geraniol might have inhibited abnormal cell proliferation occurring in skin carcinogenesis by modulating the activities of phase II detoxification agents and through its free radical scavenging potential.

5. Conclusion

From this study Geraniol shows to exhibited chemopreventive potential against in N-Nitrosodiethylamine induced hepatocarcinogenesis in Wistar Albino Rats. Thus concluding that the chemopreventive potential of Geraniol could be due to its enzymatic antioxidant and non-enzymatic properties against N-Nitrosodiethylamine (DEN) induced Hepatocarcinogenesis (HCC).

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