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RESEARCH ARTICLE

Screening of Eugenol - A Monoterpene, as an antioxidant

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Abstract

Plants are one of the most important sources of medicines. Today the large numbers of drugs in use are derived from plants. There is a strong current public interest in naturally occurring plant based remedies and dietary factors related to health and disease. Eugenol (4-allyl-2-methoxy-phenol) is a monoterpene compound pale yellow in color extracted from clove and a member of the allyl-benzene class of chemical compounds. In the current study we have aimed at exploring the antioxidant activity of eugenol in *in vivo* model along with the analysis of hepatic and cardiac markers analysis. Twenty one adult male Wistar rats, weighing 180- 250g, were randomly divided into three groups; Group 1 treated as normal control rats, Group II kept as vehicle control and Group III was administrated with 5 mg/kg b.wt of eugenol. After 30 days of experimental period the activities of blood antioxidants like Reduced Glutathione (GSH), Glutathione -s- transferases (GST), Glutathione peroxidase (GPx) and Catalase (CAT) were enhanced in eugenol treated groups along with incomparably low rate of lipid peroxidation and nitric oxide level. These results suggest that the monoterpenoid compound eugenol has the capacity to prevent oxidative damage that probably because of their antioxidant characteristics. So it can be concluded that eugenol is a very promising candidate for the design of new drugs based on its pharmacological effects of antioxidant adequacy.

INTRODUCTION

The use of plants as medicines has a long history in the treatment of various diseases. The plant derived compounds have a long history of clinical use, better patient tolerance and acceptance. In recent time there has been a remarkable increment in scientific research dealing with phytochemical to mitigate oxidative stress. Eugenol has been accepted as a non prescription drug component in traditional medicine. It is a pale yellow in color oil extracted from clove (*Syzygium aromaticum*) and a member of the allyl-benzene class of chemical compounds. Eugenol is widely used and well known for its medicinal properties (Asha et al., 2001). It is generally used as a food flavoring agent. Eugenol has been traditionally used in some parts of world as antioxidant, anti cancer, anti- inflammatory, antibacterial, analgesic and immunomodulator treatment. In view of its non-mutagenic and non-carcinogenic properties (Opdyke et al., 1975), it is generally regarded as safe by the Food and Agricultural Organization (FAO) (Chami et al., 2005) with an acceptable daily intake of up to 2·5mg/ kg body weight in humans (FAO, 1982). Previous studies have reported biological activities of eugenol including antifungal, antiallergic, antimutagenic activity and insecticidal properties (Miyazawa et al., 2001). Higher concentration of eugenol act as a pro-oxidant causing increased generation of tissue-damaging free radicals (Chogo et al., 1981; Kim et al., 2006). In this study we select the dose of 5mg/kg b.wt for acute study as per the early report by Fouad et al (2011).The present study was designed to evaluate the anti oxidant potential role of eugenol through the assessment of antioxidant system, extent

of lipid peroxidation, evaluation of nitric oxide level. In the present work, an attempt has been made to evaluate the possible *in vivo* antioxidant potential by natural monoterpene compound eugenol.

Material and Methods

2.1 Chemicals and reagents

Eugenol (99%) was purchased from HI MEDIA Laboratories Pvt. Ltd, Mumbai, India. Reduced glutathione (GSH), Oxidized glutathione (GSSG), Phenazine methosulphate (PMS), Nitroblue tetrazolium (NBT) was obtained from SIGMA –ALDRICH, Banglore, India. 2,4 –dinitro bis Nitro benzoic acid (DTMB), Nicotinamide adenine dinucleotide (NADH), Thiobarbituric acid (TBA), Nicotinamide adenine dinucleotide phosphate NADPH), and 1-chloro 2,4 dinitro benzene (CDNB), sodium nitrite, Orthophosphoric acid, Naphthyl ethylene diamine dichloride were purchased from MERK SPECIALITIES Pvt, Ltd , Mumbai , India.

2.2 Experimental design

Adult male Wistar rats weighing 180- 250g were purchased from Pharmacology Unit, Nagarjuna Herbal Concentrates Ltd, Thodupuzha, Idukki, Kerala, India. All the animals were maintained under standard laboratory conditions of temperature (25˚C) and 12 hour light and dark cycles throughout the experiment period. The rats were provided with laboratory chow and tap water *ad libitum*. The animals were divided in to three groups and each group consists of seven rats. The animals in the divided groups received oral treatments are as follows for a period of 30 days. After the experimental period all rats were decapitated and sacrificed, blood was collected for biochemical analysis.

Group I: Normal Control Rats

Group II: Vehicle control (0.1 % DMSO)

Group III: 5mg/Kg body weight of Eugenol

2.3 Methodology

2.3.1 Assay of GSH:

For the estimation of GSH (Ellman, 1959) in blood, hemolysate was prepared by adding 0.2 mL whole blood in to 2 mL distilled water and add 3mL precipitate solution mix well and after filtration take 0.2mL of clear filtrate add 0.8 mL Na_2HPO_4 read at 412 nm. Then add 0.1mL DTNB again read at 412nm.

2.3.2 Assay of glutathione -s-transferase

GST level was assayed by the method of Habig, Pabst and Jako by (1975). To the sample 0.5 M phosphate buffer (pH=6.5) and 25mM CDNB were added. After incubation at 37°C for 15 minutes, 20 mM GSH was added and change in accordance was read at 340nm for every 3 minute.

2.3.3 Assay of glutathione peroxidase

The activity of GPx was determined by the method of Rotruck et al (1975). Briefly, the reaction mixture contained 1mL of 0.4 M of sodium phosphate buffer (pH =7.0), 0.1 mL of 10 mM of sodium azide, 0.2 mL of sample 0.2 mL of glutathione, and 0.1 mL of 0.2 mM of H_2O_2. The tubes were incubated at 37^0 C for 3 minutes, then take 1 mL aliquots from it and add 4 mL metaphoshoric acid solution. After that filtering the solution and to 2 mL filtrate added 2 mL of Na_2HPO_4 and1 mL of DTNB reagent. The color that developed was read at 412 nm against a reagent blank within 2 minutes.

2.3.4 Assay of catalase

Catalase activity was measured using H_2O_2 (3%) as the substrate in phosphate buffer (pH=7.4).The reaction was started by the addition of 1 mL of serum in to it and the change in absorbance at 240 nm for 2 minutes at 30 seconds intervals (Aebi et al., 1978).

2.3.5 Assay of TBARS

TBARS activity was estimated by the method of Beuge and Aust (1978). 0.1 mL of sample was mixed with 0.2mL 0f MDA reagent, centrifuged at 1000 rpm after 15 minutes incubation in boiling water bath. Supernatant was taken and read at 535nm.

2.3.6 Determination of nitric oxide

The concentration of nitric oxide in the form of nitrate was determined using Griess reagent (1% sulphanilamide, 0.1% naphthylethylenediamine dichloride and 2% phosphoric acid). The amount of nitrate present in various samples was measured at 540 nm (Lepoivre et al., 1990).

2.3.7 Serum analysis

Aspartate amino transferase (AST), Alanine amino transferase (ALT), Lactate dehydrogenase (LDH), Creatine Kinase (CK MB) and Total Protein were detected (AGAPPE Diagnostic Ltd, Ernakulam, Kerala, India) using UV-Visible Spectrophotometer (U-5100, HITACHI HIGH TECHNOLOGIES, America, Inc.).

2.3.8 Statistical analysis

Data were obtained from repeated experiments and the results were represented as mean ± standard deviation (SD). The results obtained from the experiments were analyzed using the statistical program Origin, version 7 (Origin Lab Corporation, Northampton, USA).

Results

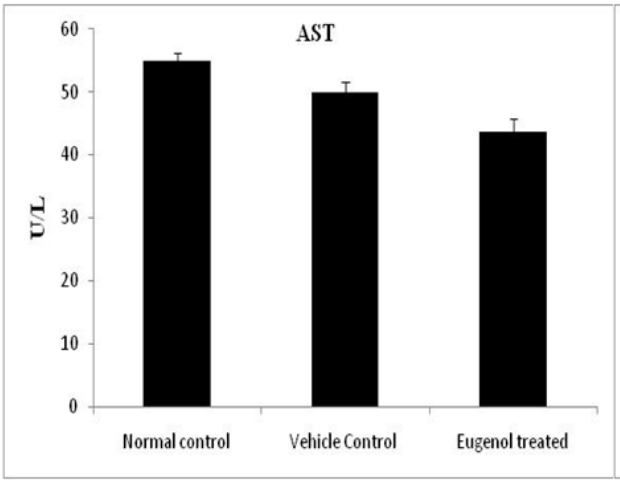

Fig.1

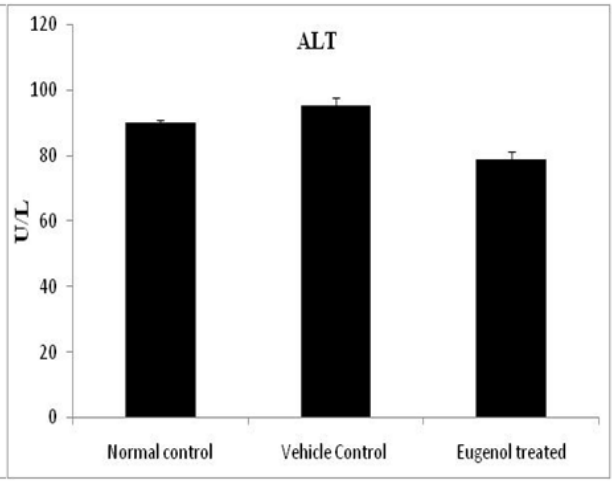

Fig.2

To assess the hepatoprotective role, serum AST and ALT levels were determined. Fig (1 & 2) shown no significant ($p < 0.05$) change in the level of AST and ALT in eugenol treated group compared with the normal control rats.

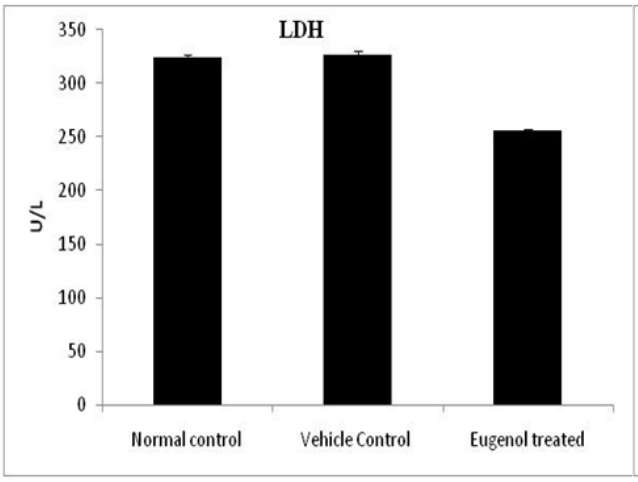

Fig.3

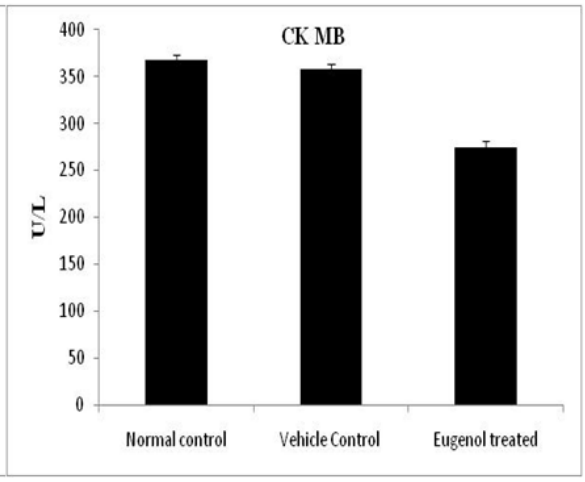

Fig. 4

In Fig (3 & 4) depicts the activities of cardiac markers LDH and CK MB activity. The data represented as mean ±SD n and in eugenol treated group showed no significant ($p < 0.05$) change in the activity of these enzymes compared with normal control ones.

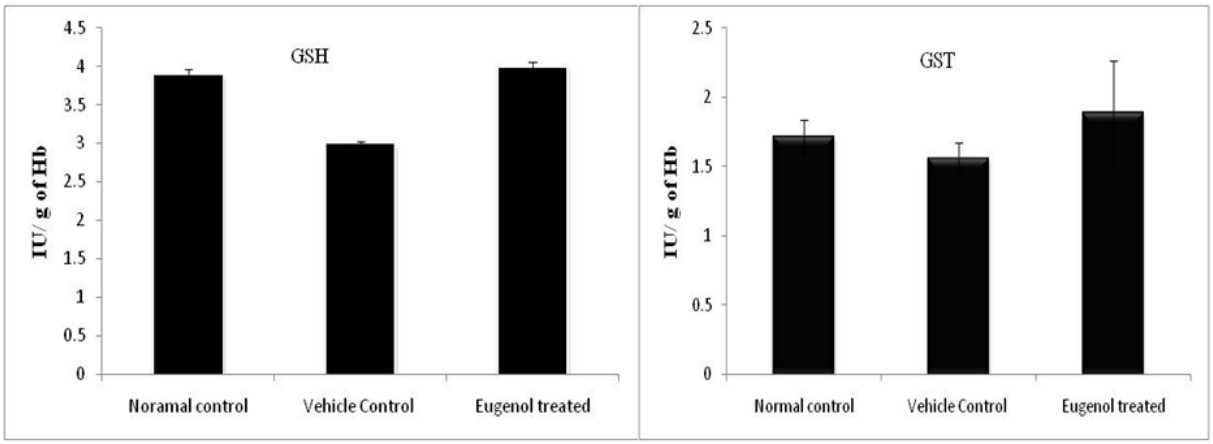

Fig.5

Fig.6

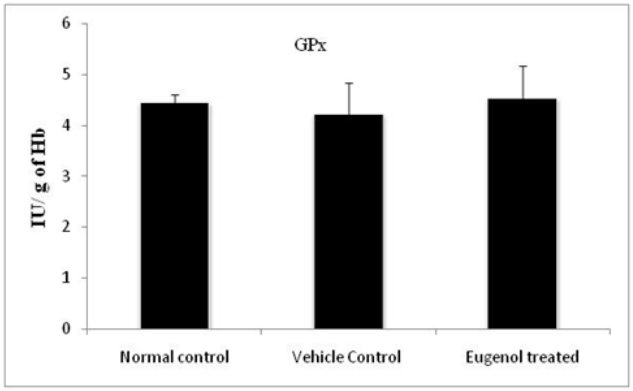

Fig.7

Data represented as mean ±SD. Free radical quenching capacity was analyzed through the level of both non-enzymatic (GSH) and enzymatic antioxidants (GST, GPx & CAT). Figure (5, 6 &7) represents, administration of eugenol (5mg/kg b.wt) caused an elevation in antioxidant level with a significant change ($p < 0.05$) eugenol treated group compared with normal control.

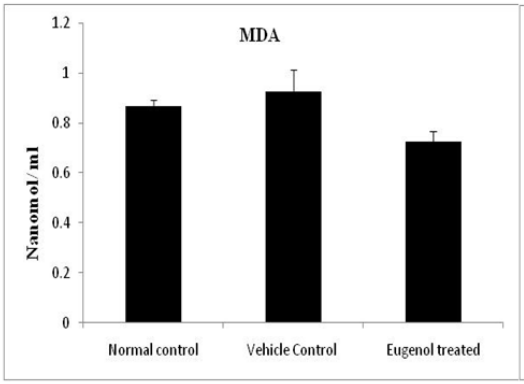

Fig.8

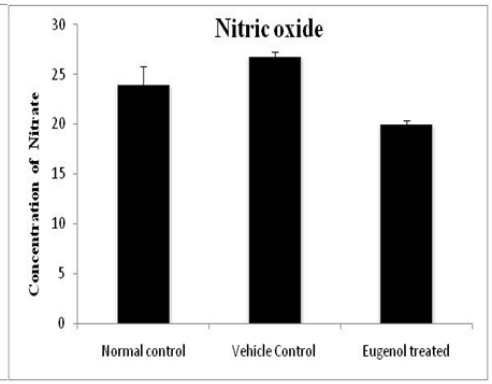

Fig.9

Data represented as mean ±SD. The level of MDA in normal control and experimental rats were illustrated in fig (8). Malondialdyhyde (MDA) was the end product of lipid peroxidation was comparatively declined during eugenol treatment. Production of nitric oxide during eugenol treatment also showed no significant change in compared with normal control rats (Fig 9).

Discussion

In the present time considerable attention has been given to free radical mediated damage in biological system and along with the search for herbal drugs with antioxidant activity for dietary intake. Antioxidants have been reported to prevent oxidative damage caused by free radicals. Oxidative stress has been associated with diverse effects on various pathophysiological conditions. The findings of our present study suggested that eugenol possessed antioxidant potential in rats. Our data showed that treatment with eugenol strengthened both enzymatic and non enzymatic antioxidant system, prevent the extent of lipid peroxidation and normalized the level of both cardiac and hepatic markers in serum.

Usually, the extent of hepatic damage is assessed by the increased level of cytoplasmic enzymes ALT and AST. Therefore ALT is more specific to the liver, and is thus a better parameter for detecting the hepatic injury. Elevated levels of serum enzymes are indicative of cellular leakage and loss of functional integrity of cell membrane in liver (Drotman et al., 1978).Our result revealed the fact that the no significance difference seen among normal control with eugenol treated but the level was diminished compared to normal rats. Efficacy of eugenol on genotoxicity on established mutagen in the liver was already reported (Rompelberg et al., 1996).

The principle of antioxidant activity is based on the availability of electrons to neutralize any free radicals. Serum enzymes are diagnostic markers because of their specificity and catalytic activity. In this study, we demonstrated that eugenol has the capacity to trap free radical. During eugenol therapy showed normalized level of both the cardiac markers LDH and CK MB. LDH is an intracellular enzyme, the increase of which in serum is an indicator of cell damage (Valko et al., 2007). No significant change in these enzymes suggested its cardiac potential capacity of phenolic compound eugenol.

The present study showed the antioxidant and radical scavenging mechanism of eugenol by antioxidant assays and analysis of lipid peroxidation. To eliminate free radicals, these cellular antioxidants play an important role and equilibrium exists between these enzymes under normal conditions.When excess free radicals are produced, this equilibrium is lost and consequently oxidative insult is established. In this study eugenol treated group shown significant (p<0.05) change in the activity of antioxidant like GSH, GST and GPx. The major role of endogenous antioxidants GSH, GPx and GST was the maintenance of redox potential (Ilavenil, 2012; Yoshida et al., 1997) and GSH might act as a substrate for GST and Gpx. GPx reduces free hydrogen peroxide to water and lipid hydroperoxides to their corresponding alcohols. There are several isozymes of GPx and GPx1 is the most abundant version, found in the cytoplasm of many mammalian tissues (Gulçin et al., 2010). It was reported that eugenol could act as a scavenger of superoxide anion and hydroxyl radicals (Rajakumar et al., 1993).

Lipid peroxidation in biological systems has long been thought to be a toxicological phenomenon. In normal physiological conditions, low concentration of lipid peroxidation products and byproducts are seen in biological system. Here in the case of eugenol treated rat showed low level of MDA compared to control rats. The byproduct lipid peroxides always alter the function of membrane proteins and lead to the production of peroxy nitrite radicals (Rajakumar et al., 1993).The effect of eugenol on lipid peroxidation and oxidation of low density lipoprotein (LDL) was studied by Baratt et al (1997). Inhibitory effects of eugenol compounds on the lipid peroxidation process were closely related to the metal reducing activity (Ito et al., 2005). So lipid peroxide marker analysis revealed that, this phytochemical compound exert any kind of alterations in oxidation of lipids which may be done by trap free radicals trap free radicals formed during normal metabolic process due to its phenol structure (Park et al., 2000).

Development of oxidative damage is closely related to the reactive nitrogen species in addition to active oxygen that is, peroxynitrite produced by the reaction of superoxide radical with nitric oxide shows potent cytotoxic effects (Ou et al., 2006; YukIlhami et al., 2011). In our study level of nitric oxide was declined in some extent as compared with the normal control which could a potentiality of natural compound eugenol as a reactive nitrogen scavenger. Eugenol's peroxy radical scavenging mechanism was extabilished (Weihua et al., 2006).

In conclusion, that eugenol possesses significant anti oxidant activity without any side effects, it may be useful in the treatment of many oxidative stress related conditions. The antioxidant potential of eugenol was efficacious on cardiac and hepatic diagnostic markers and our result confirms that the administration of eugenol

more potential against free radicals. Further studies need to explore the exact mechanism of action of this monoterpene compound on different organ systems.

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