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Antioxidant effect of eugenol in rat intestine

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The effect of eugenol on the antioxidant status of the rat intestine after short and long term (15 days and 90 days respectively) oral administration of 1000 mg/kg.b.wt (a dosage which has been reported to be highly hepatoprotective)[1] was studied. The level of lipid peroxidation products (TBARS) and the activities of glutathione peroxidase (GPx), glutathione reductase (GR), superoxide dismutase (SOD) and catalase (CAT) were found to be near normal on eugenol treatment. The level of glutathione (GSH) did not show any change on 15 days of eugenol treatment, but it was increased significantly on 90 day eugenol treatment. The activity of glutathione-S-transferases (GSTs) was increased significantly in both 15 day eugenol treated and 90-day eugenol treated groups. The results suggest that eugenol is nontoxic, protective and induces glutathione-S-transferases (GSTs) and thereby it may facilitate the removal of toxic substances from the intestine.

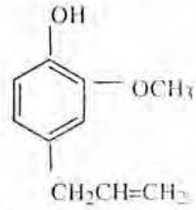
Eugenol is a naturally occurring allyl benzene and an active principle of clove, ocimum, nutmeg and cinnamon[2]. It has been used since atleast the nineteenth century, primarily as a flavouring agent in a variety of foods and pharmaceutical products and as an analgesic in dental materials[3]. Eugenol has been accepted as a non prescription drug component in traditional medicine and it is used in the treatment of flatulent colic, chronic diarrhoea and other gastrointestinal disorders[4,5] . The LD_{50} values of orally administered eugenol in mice and rats are 3000 and 2680 mg/kg body wt, respectively[6,7].

Eugenol is already known to be an antioxidant[8-10] and hepatoprotectant[11,12]. It has been reported that oral administration of eugenol increased the activities of liver detoxifying phase II biotransformation enzymes(UDP-glucuronyl transferase, glutathione-S-transferase and DT-diaphorase) in a dose dependent manner[1]. As intestine is the first target for any drug by oral administration, through which it is absorbed and enters into the blood circulation to produce its desirable effects, the present investigation is an attempt to study the non-toxic and protective nature of eugenol in rat intestine.

Materials and Methods

Eugenol, epinephrine, 1-chloro-2, 4 dinitrobenzene (CDNB), 2-thio barbituric acid, tetraphenyl butadiene, reduced glutathione (GSH), oxidized glutathione (GSSG), nicotinamide adenine dinucleotide phosphate (NADP), glucose-6-phosphate were purchased from Sigma chemical company (St. Louis, Mo USA). Olive oil was obtained from S.D. fine Chemicals Limited (India).

Eugenol (2-Methoxy-4-(2-propenyl phenol) is an allyl benzene. It is the main component of volatile oil of the clove and occurs to the extent of about 80%. It is a colourless or pale yellow liquid with a very pungent taste.



Structure of eugenol

Adult male albino rats of Wistar strain weighing 120 to 150g were purchased from the Frederick Institute of Plant Protection and Toxicology (Padappai, India). The animals were housed in a well aerated room and maintained on rat pellet diet (Lipton India Animal Feed, Bangalore) and water, *ad libitum*. The animals were divided into four groups. Each group consisted of six animals.

Group I : Control rats receiving only olive oil for 15 days.

Group II : Rats receiving eugenol in olive oil (1000 mg/kg body weight) orally for 15 days.

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Group III : Control rats receiving only olive oil for 90 days.

Group IV : Rats receiving eugenol in olive oil (1000 mg/kg body weight) orally for 90 days.

After the experimental period, the animals were sacrificed by decapitation. The intestine was removed and washed with ice-cold saline. A part of the tissue was homogenized in Tris-HCl buffer, *p*H 7.4 and the homogenate was used for analysis. The following estimations were done in the intestinal homogenate.

Total protein was estimated by the method of Lowry *et al.*[13], using bovine serum albumin as a standard. The levels of TBARS and GSH were estimated by the methods of Santos *et al.*[14], Moron *et al.*[15], respectively. SOD was assayed according to Misra and Fridovich[16] based on the inhibition of epinephrine auto-oxidation by the enzyme. CAT activity was measured by following the breakdown of hydrogen peroxide according to the method of Bergmeyer *et al.*[17], GPx was assayed using hydrogen peroxide as substrate according to the method of Rotruct *et al.*[18]. GST activity was measured using 1-chloro-2,4-dinitrobenzene as substrate according to Habig *et al*[19]., GR activity was assayed based on the oxidation of NADPH according to Doubler and Anderson[20], G6PD activity was measured following the reduction of NADP according to the method of Eeals and Kirkman[21]. Total lipids were extracted from the intestine according to the method of Folch *et al*[22] and the levels of cholesterol, phospholipids and triglycerides were estimated by the methods of Parekh and Jung[23], Fiske and Subba Rao[24], Foster and Dunn[25], respectively.

Student's t-test was applied for statistical comparison of data.

Results and Discussion

There was no significant change in the level of TBARS and in the activities of primary antioxidant enzymes, SOD and CAT. The level of GSH was found to remain normal on 15 days of treatment, but the level was increased significantly after 90 days of treatment ($P<0.05$) (Table 1).

Eugenol treatment did not show any changes in the activities of GPX, GR, G6PD after 15 and 90 days of treatment. GST activities were found to be increased significantly ($P<0.001$) after short and long term treatment of eugenol. (Table 2).

The level of cholesterol, phospholipids and triglycerides did not show any marked differences after eugenol treatment (Table 3).

The major metabolic route of eugenol is the conjugation of the free hydroxyl group, either with glucuronic acid or with sulphate. Oral administration of eugenol has already been reported to induce liver GSTs and thereby it acts as a hepatoprotectant and facilitates the removal of toxic substances. Intestine may be regarded as a tube which is constantly exposed to a number of toxic substances and its metabolites.

In the present study, the level of TBARS and the activities of intestinal antioxidant enzymes such as SOD, CAT, GX, GR, G6PD were found to remain unaltered after short and long term oral administration of eugenol.

The level of GSH which remained normal after 15-day eugenol treatment was found to be increased significantly after 90-days eugenol treatment. This

Table 1—Levels of TBARS, GSH and activities of SOD, CAT in 15-day control, 15-day eugenol treated, 90-day control and 90-day eugenol treated rat intestine.
[Values are mean ± SD for six animals in each group.]

Animals	GSH (nmole /g wet tissue)	TBARS (nmole of MDA formed/mg protein)	SOD (units/mg protein)	CAT (μmole of H_2O_2 decomposed /min/mg protein)
Group 1				
15-day control	4.47 ± 0.32	0.395 ± 0.05	3.69 ± 0.37	0.26 ± 0.06
Group 2				
15-day eugenol treated	4.64 ± 0.38^{NS}	0.380 ± 0.08^{NS}	3.78 ± 0.36^{NS}	0.27 ± 0.05^{NS}
Group 3				
90-day control	4.93 ± 0.41	0.365 ± 0.09	3.67 ± 0.49	0.25 ± 0.04
Group 4				
90-day eugenol treated	5.67 ± 0.48*	0.38 ± 0.06^{NS}	3.76 ± 0.52^{NS}	0.26 ± 0.05^{NS}

Group 2 was compared with group 1; group 4 was compared with group 3
NS - Not significant
*$P<0.05$

increase in reduced GSH may be due to the increased demands for the activity of GST which utilise GSH as substrate. GSH is the major component of the endogenous non protein sulphydryl pool and it binds to reactive free radicals and may influence the physical properties of mucus, since its subunits are joined by disulphide bridges. According to Boyd *et al.*[26], Diethyl maleate, an agent that markedly depletes gastric GSH, causes severe gastric ulceration. Thus GSH which is increased significantly after 90 day eugenol treatment may give cytoprotection to the intestine and this may be responsible for its anti-inflammatory property.

The activity of GSTs was increased significantly ($P<0.001$) in both 15-day eugenol treated and 90-day eugenol treated groups. It has been reported that oral administration of eugenol induces phase II biotransformation enzymes such as GST and UDP-glucuronyl transferase in rat liver[1,27]. In mouse also, oral administration of eugenol enhanced the GST activity in liver and small intestine[28]. The present results also show a similar statistically significant ($P<0.001$) increase in GST activity in the intestine of eugenol treated groups compared to their respective control groups, thereby confirming its nature as an inducer of GST in intestine also.

The membrane lipids which are responsible for functional integrity of the membrane were found to be unaltered in both 15 day eugenol treated and 90 day eugenol treated groups. No significant changes in the levels of cholesterol, phospholipids and triglycerides were observed. Eugenol has been reported to lower or tends to lower cholesterol and triglycerides in liver[27]. The hypocholesterolemic effect of eugenol in liver may be due to the enhanced conversion of hepatic cholesterol to bile acid, and this may justify its use in digestive disorders.

In conclusion, the present study shows the non toxic and protective nature of eugenol in rat intestine.

Table 2—Activities of GPx, GST, G6PD and GR in 15-day control, 15-day eugenol treated, 90-day control and 90-day eugenol treated rat intestine.
[Values are mean ± SD for six animals in each group.]

Animals	GPx (μg of GSH consumed / min/mg protein)	GST (μmole of CDNB conjugated/ min / mg protein)	G6PD (U/mg protein)	GR (μmole of NADPH utilised/min/mg protein)
Group 1				
15-day control	35.94 ± 2.44	8.06 ± 0.87	2.18 ± 0.37	25.40 ± 0.79
Group 2				
15-day eugenol treated	36.25 ± 2.97^{NS}	11.66 ± 0.95***	2.24 ± 0.32^{NS}	25.96 ± 0.92^{NS}
Group 3				
90-day control	35.84 ± 2.43	8.03 ± 0.80	2.62 ± 0.30	25.12 ± 0.88
Group 4				
90-day eugenol treated	36.63 ± 2.65^{NS}	13.53 ± 0.97***	2.78 ± 0.39^{NS}	25.45 ± 0.78^{NS}

Group 2 was compared with group 1; Group 4 was compared with group 3
NS - Not significant
*** $P<0.001$

Table 3—Levels of cholesterol, phospholipids and triglycerides in 15-day control, 15-day eugenol treated, 90-day control and 90-day eugenol treated rat intestine.
[Values are mean ± SD for six animals in each group.]

Animals	Cholesterol (mg /g fresh tissue)	Phospholipids (mg/g fresh tissue)	Triglycerides (mg/g fresh tissue)
Group 1			
15-day control	5.86 ± 0.51	27.86 ± 2.43	3.24 ± 0.38
Group 2			
15-day eugenol treated	6.06 ± 0.42^{NS}	27.92 ± 2.62^{NS}	3.35 ± 0.42^{NS}
Group 3			
90-day control	5.98 ± 0.77	28.55 ± 2.73	3.62 ± 0.48
Group 4			
90-day eugenol treated	6.26 ± 0.64^{NS}	28.87 ± 2.04^{NS}	3.72 ± 0.32^{NS}

Group 2 was compared with group 1, group 4 was compared with group 3.
^{NS} Not significant

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