Research Article

Effect of Sodium Fluoride (NaF) Exposure on Heart: Protective Effect of Eugenol with Reference to the Lipid Metabolic Profiles

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ABSTRACT

The aim of our study is to investigate the anti-hyperlipidemic effect of eugenol, a bioactive compound of *Ocimum sanctum* in the NaF intoxicated fluorosis induced albino male rats of wistar strain albino rats. *Ocimum sanctum* (OS) has been used in the Indian system of traditional medicine for the treatment of diabetes, liver disorders and heart problems. However, the bioactive compound of *Ocimum sanctum*, eugenol with reference to fluorosis was not carried out till date. Male rats were administered with eugenol for 60 days. The lipid metabolic profiles like MDA, TC, TG, LDL, VLDL and HDL are estimated in the plasma of all groups. The fluorosis markers calcium and phosphorus are also estimated. MDA, TC, TG, LDL, VLDL levels are elevated while HDL levels are depleted in NaF treated rats. Calcium levels decreased and phosphorus levels increased in NaF administered rats. However, eugenol supplementation for 60 days normalized all the lipid metabolic profiles in NaF rats. The fluorosis markers also came to near normalcy in eugenol treated rats. This shows the anti-lipidemic properties of eugenol.

Keywords: NaF, eugenol, lipids, rats.

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INTRODUCTION

Currently, fluoride pollution is a worldwide problem as the fluoride levels in drinking water above the tolerable limit in several countries such as China, Sri Lanka, West Indies, Holland, Italy, and India.[1]In India, fluoride levels in drinking water are also above the permissible limit in wide areas of the states of Andhra Pradesh, Tamil Nadu, Karnataka, Punjab, Bihar, Haryana, Kerala and West Bengal.[2] The international standard of permissible limit of fluoride in drinking water has been established at 1.[3] Fluoride is a common element in our daily life as it is natural mineral component of drinking water, tea, meat, beverages. It is often added to dental products because of its ability to prevent dental caries or cavities. However, excess intake of fluoride can result in fluorosis that causes not only dental and skeletal damage but also problems in the central nervous system such as brain damage, Alzheimer syndrome, thyroid disorder, DNA damage, gastro-intestinal irritation, and kidney function disorder.[4]

The most common manifestations that have been reported arising from chronic exposure of fluorides are skeletal, dental fluorosis, arthritic pain, osteosclerosis, and crippling fluorosis.[5] Fluoride intoxication results in neurologic damage, primarily in the impairment of both cognition and memory.[6] Fluoride intoxication also results in metabolic disorders, hepatitis, renal toxicity, muscular atrophy, decalcification and fragility of bone, and reproductive disorders.[7-9]

Numerous medicinal plants have been identified that exhibit anti-oxidative and anti-hyperglycemic activities. Among them, *Ocimum sanctum* L. has got paramount importance in folklore and traditional medicinal practices such as Ayurveda, Siddha and Unani. OS has been recommended for the treatment of a number of diseases.[10] Therapeutic value of *Ocimum sanctum* L. to treat a number of diseases including bronchitis, diarrhea and dysentery. OS has antioxidant, antibiotic, hypocholesterolemia, and hypoglycemic as well as hypotensive activities. [11]

The objective of the study was to investigate the anti-fluorosis, anti-lipidemic activity of eugenol, a bioactive compound of *Ocimum sanctum* in NaF induced fluorosis rats. This is the first reported data on the effect of eugenol in fluorosis induced rats.

MATERIALS AND METHODS

Chemicals and reagents

All chemicals used were of analytical grade and purchased from Sigma–Aldrich, Merck, India. All chemicals and reagents were used without further purification.

Selection and collection of plant material

Leaves of OS were collected from Seshachalam hills of Tirupati, Andhra Pradesh, India and identified by the Dr. Madhav Chetty, Department of Botany, SV University, Tirupati. Voucher specimen of *Ocimum sanctum* No 1183 was deposited in the Botany department.

Extraction of Eugenol from *Ocimum sanctum*

The extraction of eugenol was carried out with batch extraction setup. It consists of a 500 ml reactor in which extraction is to be carried out; a turbine type four blade agitator connected with motor (REMI, maximum speed of 1200 rpm) was used.

Grouping of Animals

The study design was approved by the animal ethical committee (Resolution No.10/08/a/CPCSCA/IAEC/SVU/09-10/ZOOL/KRS/Dt.25.09.2009). After 7 days of acclimatization, the rats were divided into five groups of six rats in each group and treated as follows.

Group I – Normal Control (NC): Six rats received saline via orogastric tube for a period of 60 days.

Group II – Eugenol Control (EGt): Six rats received *Ocimum sanctum* bioactive compound (10mg/kgBW) for 60 days.

Group III – NaF treatment (Na F): Six rats received the sodium fluoride (10mg/kgBW) dissolved in water for 60 days.

Group IV – NaF + Ocimum treatment (NaF+EGt): Six rats received fluoride and eugenol as described in group 3 and group 2for a period of 60 days.

Group V –Na F+ Vitamin E treatment (NaF+VitE): Six rats received fluoride and Vitamin E for a period of 60 days.

The animals were sacrificed by cervical dislocation and the blood was collected for biochemical investigations after completion of 60 days of treatment and with a 24 hrs. of brief time lapse of the last treatment.

Estimations of Lipid metabolic profiles

Blood was collected from the abdominal vein to determine lipid profile parameters such as total cholesterol (TC), triglyceride (TG), low-density lipoprotein cholesterol (LDL), very low-density lipoprotein cholesterol (VLDL) and high-density lipoprotein cholesterol (HDL). Fluorosis markers like calcium and phosphorus levels are estimated in the plasma all the groups of rats.

Statistical analysis

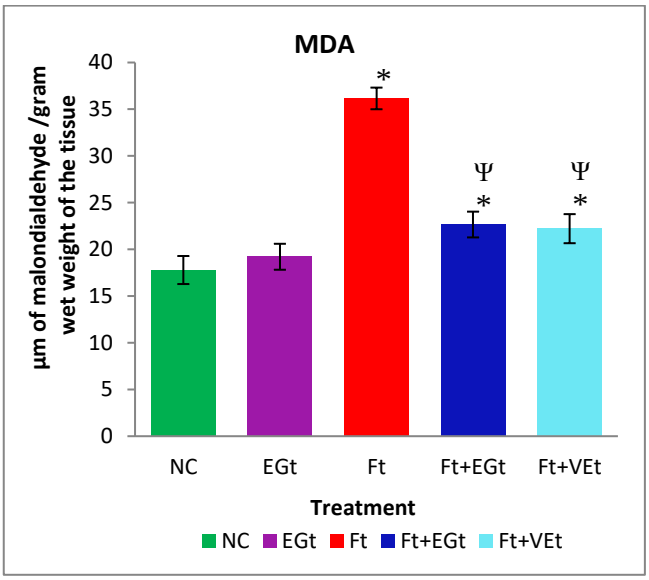
The data are expressed as mean values with their Standard Deviation (SD). Readings of the five different groups were compared using one-way ANOVA analysis with Dunnett's multiple comparison Test. Statistical analysis was performed using SPSS (Version 13.5; SPSS Inc., Chicago, IL, USA). Using M.S. Office, excel software. The data has been analyzed for the significance of the main effects (factors) and treatments along with their interactions. Differences were considered significant at $p < 0.001$.

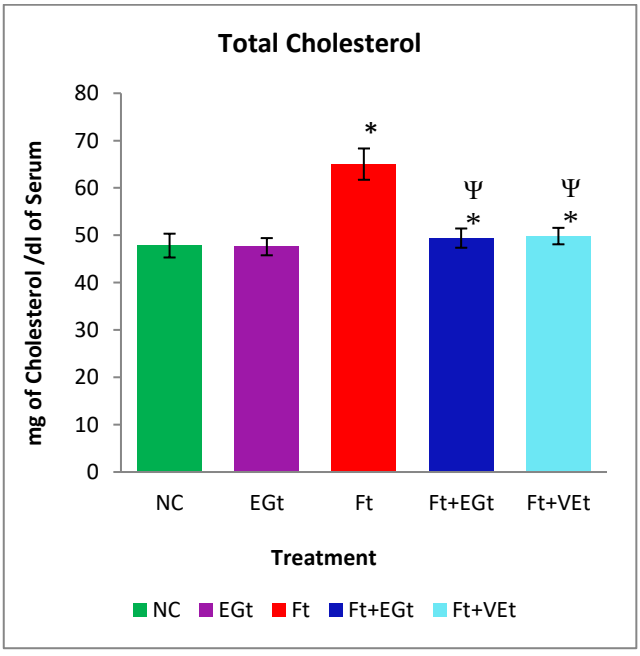
RESULTS

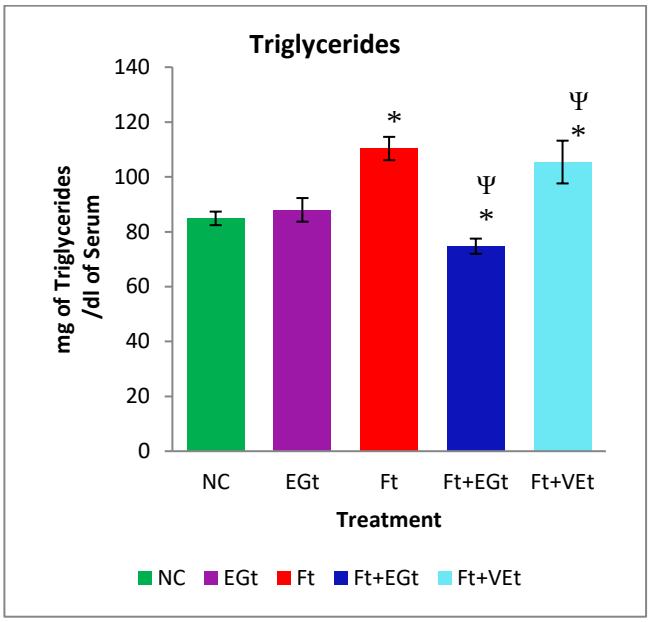
Effect of Eugenol on Lipid metabolic profiles in Sodium Fluoride intoxicated rats

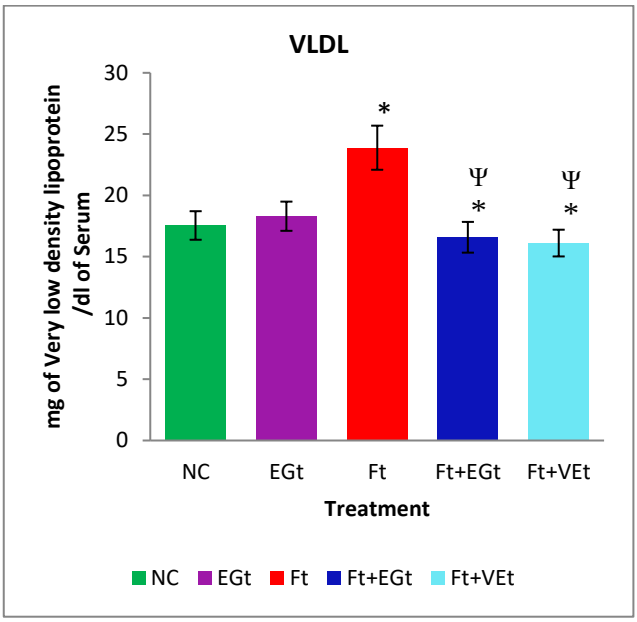
In the present investigation, MDA, TC, TG, LDL, VLDL levels are significantly increased and HDL levels are decreased in Na F intoxicated rats, but eugenol supplementation could decrease all these lipid metabolic profiles except HDL cholesterol. This shows the anti-hyperlipidemic effect of eugenol (Figure 1).

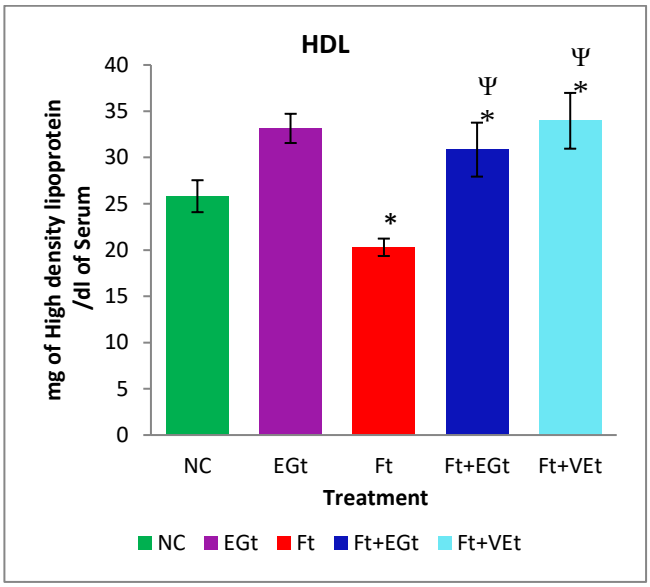
Figure 1: The Impact of Eugenol on Lipid metabolic profiles in NaF intoxicated rats. Data are expressed as means ± SD (n = 6). * The values are significant compared to the following: control (* p < 0.001), Fluorosis (Ψp < 0.01) (Dunnett's multiple comparison tests).

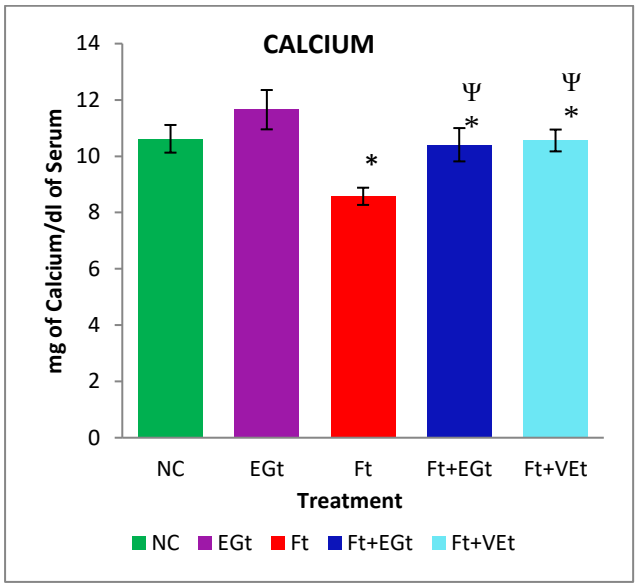


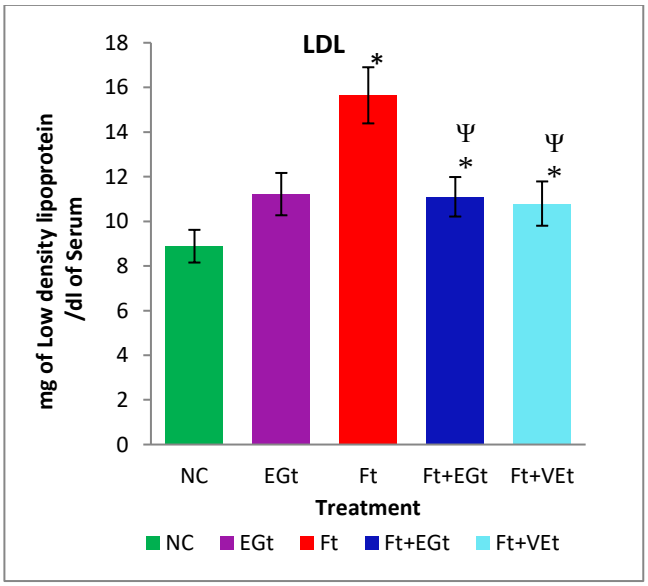


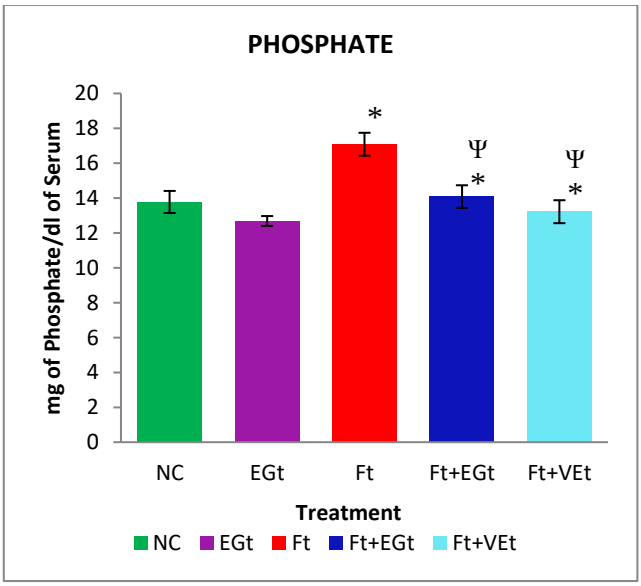












Effect of Eugenolon fluorosis markers in NaFintoxicated rats

In the current study, we observed that calcium levels are depleted and phosphorus levels are elevated influoride intoxicated rats. However, with eugenol treatment for 60 days modulated the fluorosis markers in all experimental treated rats.

DISCUSSION

The present study was done to know the pharmacological action of eugenol in NaF intoxicated rats. Bioactive compounds of *Ocimum sanctum* reported to possess antitussive, hypoglycemic, antioxidant, antistress, anti-ulcerative, anti-inflammatory, antiasthmatic, immune stimulatory and neuroprotective effects.

Sufficient evidence has demonstrated that fluoride produces deleterious effects in skeletal, dental and soft tissues. There are documentary evidences concerned with the mechanism of bone and dentalfluorosis.[12] But it is not clear of how fluoride interferes with soft tissues. However, some of the following viewpoints may be very helpful for us to understand the mechanism of fluorosis in soft tissues.

Lipid related abnormalities are most common in toxicity subjects. Alterations in lipid metabolism have been reported in NaF intoxicated rats. In this study, we observed elevated levels of MDA, total cholesterol, LDL cholesterol, VLDL cholesterol, triglycerides in NaF rats. This may be due to the lipid peroxidation process that occur in plasma membrane, microsomes and mitochondrial membranes in fluorosis subjects.[13]Several studies declared that increased production of ROS is an important mechanism in fluoride-induced toxicity in experimental animals.

Exposure to fluoride has been reported to induce oxidative stress in vivo and in vitro. Oxidative stress is considered to play an important role in the pathogenesis of fluorosis and excess ROS production liberated from peroxisomes and mitochondria can directly inflict direct damage to structural polar lipid molecules of various cellular membranes. MDA, end product of lipid peroxidation in tissues, is also an indicator of oxidative stress. Lipid peroxidation is a free radical-mediated process that occurs following oxidative stress. Alterations in lipid metabolism maybe responsible for the altered MDA, TC and TG levels in fluorosis condition. It appears that enzymes inhibited by fluoride, such as triglyceride lipase, unspecific esterase and pyrophosphates. Also, the obtained results of hyperlipidemia may be attributed to an increase in the synthesis of fatty acids in the liver or possibility due to incidence of liver cholestasis 2000.[14]

Many reports suggested that the abnormal activities of lipase enzymes seem to be one of the chief factors responsible for the rise in plasma triglycerides and total cholesterol. However, with eugenol supplementation, TG an TC levels are depleted in NaF treated rats by preventing lipid peroxidation. This is attributed to the anti-lipidemic property and free radical scavenging activity of eugenol.

In our study, we reported that LDL, VLDL are elevated and HDL levels are depleted in NaF treated rats when compared to control. This dyslipidemia manifestations are due to decreased activity of lipoprotein lipase. Increased VLDL levels in NaF rats are due to increased concentration of free fatty acids.

Lipid disorders, most often encountered in fluorosis diseases also include increased levels of LDL and VLDL.[15] Eugenol supplementation may elevate the activity of lipoprotein lipase and decrease in LDL, VLDL in NaF treated rats. Moreover, with eugenol treatment HDL levels are increased in NaF rats. It could be understood rise in levels of lipoprotein lipase and HDL prevents the formation of coronary artery plaques and cardiac ischemia.[16]Hence, eugenol may be considered as a candidate for prevention of cardiovascular disorders since it promotes anti-lipidemic factors like HDL and LDL. In conclusion, our results revealed the hypolipidemic effect of (−)-eugenol that was evidenced by all the lipid metabolic profiles MDA, TC, TG, LDL, VLDL, HDL return to normalcy with eugenol supplementation in fluorosis rats.[15] (Figure 1).

In our study, we reported that calcium levels decreased and phosphorus levels increased in NaF rats. This may be due to alterations in metabolism of calcium and phosphorus. The calcium in the blood is transported to the intercellular fluid after which it is deposited in bones and teeth. However, with eugenol treatment for 60 days, these fluorosis markers normalized to near normal levels.

In our study, we reported that eugenol ameliorated the lipid metabolic profiles in sodium fluoride induced toxicity rats. Hence, we concluded that eugenol of *Ocimum sanctum* can be used as anti-lipid agents in many diseases.

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