



# CARDIO PROTECTIVE PROPERTY OF NATURAL COMPOUND EUGENOL IN FLUORIDE INTOXICATED MALE RATS WITH RESPECT TO ANTIOXIDANT ENZYMES

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## ABSTRACT

Eugenol is a medicinally important antioxidant phenolic compound. This plant compound has been used as ameliorative agent against variety of severe ailments. The aim of this research was to see whether the Eugenol has any cardio protective property. We tested eugenol on male albino rat's cardiac tissue suffering from fluoride toxicity with reference to antioxidant enzymes. The results showed that antioxidant enzymes such SOD, CAT, GPx and GR levels were considerably higher in the eugenol treated fluoride intoxicated group, but they were lower in the fluoride alone rat group. In conclusion, eugenol displays cardio protective effects in the cardiac tissue via boosting endogenous antioxidant enzymes. Future clinical study into the mechanism of action of eugenol under fluoride intoxication will confirm the current findings.

**KEYWORDS:** Antioxidant enzymes, cardio protective, fluoride intoxication, eugenol

## INTRODUCTION

Fluorine is an element that does not occur in the elemental state in nature because of its high reactivity. Many studies revealed that the long-term fluoride intake has damaging effects on the heart. Association between fluoride endemicity and prevalence of cardiac complications has been observed among adult males (Amini et.al.,2011). Diastolic dysfunction due to chronic fluoride exposure was observed in fluoride endemic areas and also noticed in elevation of the risk of heart failure (Varol et.al.,2010). Oxidative stress is known to be a key factor to heart failure and ischemic heart disease (Kiyuna et.al., 2018). Fluoride interacts with cellular systems and induce oxidative stress by induce the production of reactive oxygen species (ROS) and by modulating intracellular redox homeostasis, lipid peroxidation, and alteration of gene expressions were studied (Chattopadhyay et. al., 2011).

Eugenol (4-allyl-2-methoxy-phenol), the principle bioactive compound of *Syzygium aromaticum*, *Ocimum sanctum*, *Pimenta racemose* is an antioxidant, used as a food-flavouring agent and as an analgesic. Eugenol, a methoxyphenol with a short hydrocarbon chain, found in bay leaves, allspice, and the oil of cloves that originate from the *Syzygium* species (Kollmannsberger and Nitz, 1994). Eugenol antioxidant activity has been evidenced by the DPPH assay (Teresa et.al., 2006). Eugenol possesses the antioxidant property by virtue of its phenolic hydroxyl group that donates electrons to quench free radicals. Hence it could preserve the enzymatic and nonenzymatic antioxidant systems in the myocardium and prevent lipid peroxidation. Eugenol could prevent electrophysical alterations and electrolyte imbalances and restore QT interval to normal (Binu et.al.,2017). Eugenol has attenuated isoproterenol-induced cardiac apoptosis by increasing intracellular calcium and oxidative stress (Choudhary et.al.,2006). Eugenol compounds have a methoxy phenolic structure

similar to capsaicinoids, inhibited iron-mediated lipid peroxidation and copper dependent LDL oxidation. The antioxidant action of eugenol compounds was analysed by its radical-scavenging activity and on the basis of the protective effect on metal-mediated lipid peroxidation. (Ito *et.al.*,2005). The antioxidant activity of eugenol was either equal to or better than the activities of TBHQ and BH. The presence of the 4-OH group in eugenol may be responsible for the antioxidant activity. Eugenol has shown good antioxidant activity at 10- $\mu$ M concentration (Kelm *et.al.*, 2000). Based on literature review, we aimed at identification of antioxidant activity of eugenol under fluoride intoxication, in the present study.

## MATERIAL AND METHODS

### Care and Maintenance of Experimental Albino Rats:

Thirty healthy adult male Wister strain albino rats (weighing 200 $\pm$ 20g) were purchased from M/s. Sri Venkateswara Enterprises, Bangalore, India. The animals were kept in cages made up of Polypropylene with top stainless-steel grill. Six rats were accommodated in each cage and maintained under controlled laboratory conditions (Photoperiod of 12 hours light/ 12 hours dark cycle and Temperature 25 $\pm$ 2  $^{\circ}$ C). The rats were given a standard pellet diet (Purchased from Lipton Animal feed Ltd. Pune, India) and provided drinking water ad libitum during the complete experimental period. All the experiments were carried out according to the guidelines of the Institutional Animal Ethical Committee. (Resolution No: 09/(i)/a/CPCSEA/IAEC/SVU/ZOOL/KSR/Dt. 08-07-2012).

### Selection of Age Group:

Based on the growth and physiology of the animal, four (4) months aged male Wister albino rats were considered as "Adults" and selected for the present experiment. A definite table regarding the age and life expectancy of various strains of laboratory animals was published in the book, entitled "International care and treatment of rabbits, mice, rats, guinea pigs and hamsters" by W.B. Saunders Co., Philadelphia, USA. Age-related changes in 2 weeks, 2.5, 10 and 23 months of Wistar strain rats were clearly explained by Jang *et al.*, (2001).

### Procurement of Chemicals:

All the chemicals used in the experiments were Analar Grade (AR) and purchased from Sigma Aldrich Corporation (St. Louise, Missouri, USA) Merck (Mumbai, India), Ranbaxy (New Delhi, India). "Barnstead Thermolyne" water purification plant was used to get nano-pure water. "Remi cooling high speed centrifuge" was used for centrifugation of the tissue homogenate. Shimadzu UV-1800 Spectrophotometer was used for measuring the optical density values and obtained results.

### Grouping of Animals:

The rats of same age were selected and segregated randomly into five groups of six rats and given treatment as follows:

- 1. Group I: Normal Control (NC):** Six rats received saline (0.9% NaCl) through orogastric tube for 60 days.
- 2. Group II: Ocimum-Bio active compound, Eugenol Control (EGt):** Six rats received Eugenol (10 mg/kg BW) for 60 days.
- 3. Group III: Na F treatment (Ft):** Six rats received the sodium fluoride (NaF) (10 mg/kg BW) dissolved in drinking water for 60 days.
- 4. Group IV: NaF + Ocimum Bioactive compound (Eugenol) (Ft + EGt):** Six rats received NaF (10 mg/kgBW) dissolved in drinking water and Eugenol (10 mg/kgBW) for 60 days.
- 5. Group V: NaF + Vitamin E treatment (NaF + VEt):** Six rats received NaF and Vitamin E (200 mg/kgBW) for 60 days.

### Isolation of heart:

After 60 days of treatment period was over, the animals were sacrificed by cervical dislocation. The heart tissue was excised and washed with ice-cold normal saline and stored in deep freezer at -80 $^{\circ}$ C for biochemical assay of antioxidant enzymes.

### Estimation of Antioxidant enzymes in Cardiac tissue:

Superoxide dismutase (SOD) activity was evaluated by the method of Misra and Fridovich (1972) at 30°C. The heart tissue was homogenized in chilled 50 mM phosphate buffer (pH 7.0) containing 0.1 mM EDTA to give 5% homogenate (w/v). The homogenates were centrifuged at 10,000 rpm for 10 min at -4°C in cooling high-speed centrifuge. The supernatant part was segregated and used for enzyme assay in both standard and experimental tubes. 200µl of tissue sample was added to 1760µl of 0.05 M carbonate buffer which contains 0.1 mM EDTA at pH 10.2 at which SOD inhibits autooxidation of epinephrine. Then 40 µl of 30 mM epinephrine (in 0.05% acetic acid) was added to the above mixture and measured the OD values at 480 nm for 4 min on a Shimadzu UV-1800 Spectrophotometer. Activity expressed in terms of units and the amount of enzyme that inhibits the oxidation of epinephrine by 50%, which is equal to 1 unit.

Catalase activity was estimated by applying the method of Aebi (1984), with slight modifications. 5% homogenate (weight/volume) of cardiac tissue was prepared in ice cold 50 mM phosphate buffer (pH 7.0) containing 0.1 mM EDTA. The homogenates were centrifuged at 10,000 rpm for 10 min at -4 °C. The supernatant part was separated and used as enzyme source. 200 µl of tissue homogenate was taken into a test tube and 20 µl of 100% Ethyl alcohol was added to it. The reaction mixture was kept in an ice bath for 30 minutes. Then the tubes were kept at room temperature till the reaction mixture attain normal room temperature and added 20 µl of Triton X-100 RS. In a cuvette containing 400µl of phosphate buffer and 100 µl of tissue extract was added 0.5 ml of 0.066 M Hydrogen Peroxide (in phosphate buffer) and decreases in optical density measured at 240 nm for 60s in a UV-Vis Double beam spectrophotometer. The molar extinction coefficient of 43.6 M cm<sup>-1</sup> was used to determine the Catalase activity. One unit of activity is equal to the moles of H<sub>2</sub>O<sub>2</sub> degraded / mg protein/min.

Se-dependant Glutathione peroxidase (Se-GSH-Px) activity was measured by the modified method of Flohe and Gunzler (1984). 5% (w/v) of cardiac tissue homogenate was prepared in 50 mM phosphate buffer (pH 7.0) containing 0.1 mM EDTA. The homogenate was centrifuged at 10,000 rpm for 10 min at -4 °C in cooling high-speed centrifuge. The supernatant was used as an enzyme source. The reaction mixture contains of 0.5 ml of phosphate buffer, 0.1 ml of 0.01 MGS (reduced form), 0.1 ml of 1.5 mM NADPH and 0.1 ml of glutathione reductase (0.24 units). 0.1 ml of tissue homogenate was added to the reaction mixture and incubated at 37 °C for 10 min. Then 50 µl of 12 mM t-butyl hydroperoxide was added to 0.45 ml of the tissue reaction mixture and measured at 340 nm for 3 min. The molar extinction coefficient of 6.22 X 10<sup>3</sup> M cm<sup>-1</sup> used to determine the activity of glutathione peroxidase. One unit of activity is equal to the mM of NADPH oxidized / mg protein / min. The activity of the enzyme was expressed in µ moles of NADPH oxidized / mg protein / min.

Glutathione reductase (GR) activity was assayed by the method of Carlberg and Mannervik (1985) with slight modifications. The cardiac tissue was homogenized (5% w/v) in 50 mM phosphate buffer (pH 7.0) containing 0.1 mM EDTA. The homogenates were centrifuged at 10,000 rpm for 10 min at -4°C in cooling high speed centrifuge. The separated supernatant part was used as enzyme source. NADPH (50 µl, 2 mM) in 10 mM Tris buffer (pH 7.0) was added to the cuvette containing 50 µl of GSSG (20 mM) in phosphate buffer (0.5 M, pH 7.0 containing 0.1 mM EDTA) and 0.8 ml of phosphate buffer. The tissue extract (0.1 ml) was added to the NADPH – GSSG buffered solution and take OD values at 340 nm of 3 min. The molar extinction coefficient of 6.22 X 10<sup>3</sup> M cm<sup>-1</sup> was used to determine glutathione reductase activity is equal to the mM of NADPH oxidized / mg protein / min. The activity of the enzyme was expressed in µ moles of NADPH oxidized / mg protein / min.

The data obtained was expressed as Mean values with their SD. Readings of the six different groups were compared using one-way ANOVA analysis with Dunnett's multiple comparison tests. 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.

### RESULTS AND DISCUSSION

In the present research work, SOD activity levels were measured in experimental animal rats. SOD levels were significantly lower in the Ft group than NC, while SOD levels were significantly higher in Ft+Egt rats when compare to Ft (Fig.1). Treatment of Eugenol to flouride rats confirmed a greater enhancement in SOD activity relative to Flouride intoxicated group and a slight decrement when compared to standard Vitamin E treatment. When compared to NC, there were no changes in the Egt group.

SOD plays a critical role in the body's antioxidant defense in contradiction of oxidative stress. The enzyme is an effective treatment for illnesses caused by reactive ROS by converting superoxide anion free radical (O<sub>2</sub><sup>-</sup>) into oxygen and hydrogen peroxide (Kangralkar et al., 2010; Yasui and Baba, 2006). It was found that oxidative stress is linked to numerous cardiac disorders, including myocardial infarction, peripheral vascular disease, and stroke, while, these conditions were get normal with supplementation of antioxidants (Farías et al., 2017). These radicals target the cardiac antioxidant system as well as biomolecules (carbohydrates, lipids, proteins, and DNA), causing cardiac tissue damage. Generally, superoxide radical is produced in the heart tissue from the mitochondrial electron transport chain. To decrease reactivity, antioxidants in the mitochondria, such as SOD and glutathione are quickly breakdown or sequester superoxide radicals. High numbers of mitochondria in cardiac tissue are required for proper function, but cardiac dysfunction can arise from a reduction in mitochondrial antioxidant ability (Sánchez-Villamil et al., 2016; Rathore et al 2008).

In this study, fluoride intoxicated rats have been exhibited the reduced activity of SOD in heart tissue. Fluoride supplemented pigs had lower levels of SOD in their hepatic tissue in a previous research. In a separate research, mice given fluoride had a detrimental effect on the testes and epididymis via decreasing SOD levels (Zhan et al., 2006; Sun et al 2018). Wistar strain male albino rats exposed to high fluoride levels in their drinking water for two generations enhanced oxidative stress via lowering SOD levels in cardiac tissue (Basha et al 2011). Hence, fluoride is toxic in inducing oxidative stress by reducing SOD activity. Present study results also correlate with the all previous studies.

Increased levels of SOD were detected in the Ft+Egt experimental group of this study, compared to fluoride-intoxicated rats that eugenol compound treatment to the fluoride rats increased the SOD activity. It was discovered that rising quantities of free radicals reduce the antioxidant enzyme system in the body, but free radical scavengers reduce radicals, allowing the antioxidant enzyme system to increase. In an *in vitro* ABTS test, eugenol decreased free radical-induced lipid peroxidation and protected liver tissue against lipid peroxidation in CCl<sub>4</sub>-induced hepatotoxicity (Nagababu et al., 2010). It is obvious that eugenol reduces the number of free radicals in the body during sickness. Similarly, eugenol may scavenge free radicals generated by fluoride in the current research, therefore increasing the SOD activity. Similar results were observed in a study, cisplatin caused oxidative stress in the testicular tissue, which reduced SOD enzyme activity, but raised SOD levels in the cisplatin group when administered with eugenol (Ekinçi Akdemir et al., 2019). In the same manner, Kumaravelu et al. (1996) discovered that eugenol has antioxidant action in CCl<sub>4</sub>-induced erythrocyte damage *in vivo*, and that eugenol enhanced SOD activity in red blood cells whereas CCl<sub>4</sub> lowered it. Eugenol activity was somewhat lower than the standard vitamin E, in this study because vitamin E is potential antioxidant agent. It was proven by huge scientific reports (Rizvi et al 2014). It is positive entity that eugenol acted SOD acceleration property which is very near to the vitamin E.

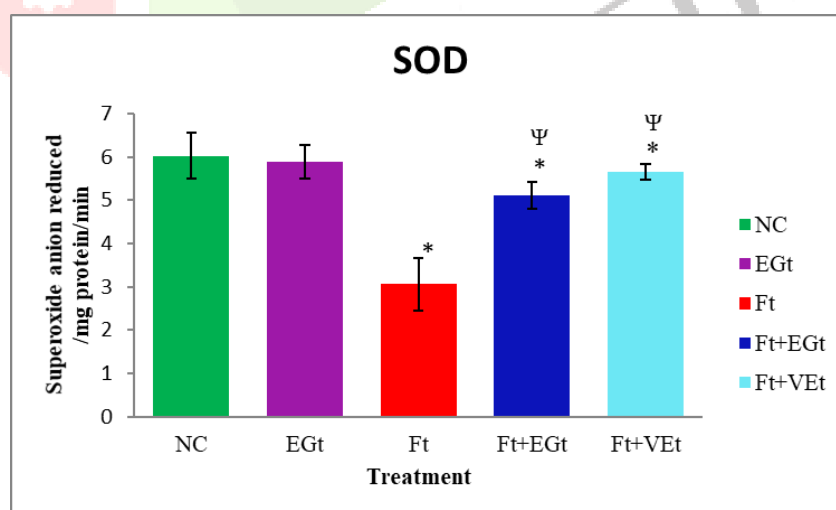


Fig.1. Effect of eugenol on SOD activity in the cardiac tissue of male albino rats. The values are significant compared with normal control (\*p<0.05).



In addition to SOD, CAT activity levels were measured in experimental animal groups. CAT levels were significantly lower in the Ft group than NC, while CAT levels were significantly higher in Ft+Egt rats when compared to Ft (Fig.2). Treatment of Eugenol to fluoride rats confirmed a greater enhancement in CAT activity relative to fluoride intoxicated group and a slight decrement when compared to standard Vitamin E treatment. When compared to NC, there were no significant changes have been observed in the Egt group.

A variety of inhibitors including sulfide, hydroxyl amine, fluoride, cyanide, azide and acetate are decrease catalase's enzymatic activity. These chemicals are thought to substitute an aquo ligand in the prosthetic group that inhibits hydrogen peroxide catalysis (Schonbaum and Chance, 1976). Fluoride inhibited catalase activity *in vitro* at 1 mg/L, according to a Clark-type oxygen electrode sensor research (Stein and Hain, 1995). When ingested in abundance, fluoride is known for causing harm to key organs and systems, including the cardiac tissue, in addition to dental and skeletal fluorosis (Cicek et al., 2005). Fluoride interferes with soft tissues by blocking a variety of enzymes, ultimately resulting in the formation of free radicals. In the present study also, fluoride ingested rats exhibited changes in antioxidant enzyme CAT in heart tissue. Our findings were also consistent with a previous study that rat puffs exposed to 200 ppm NaF by the mother's milk throughout lactation and directly exposed through fluoride water in the post-suckling time frame for 3 months had lower antioxidant activity and decreased catalase enzyme in heart tissue (Basha and Sujitha, 2011).

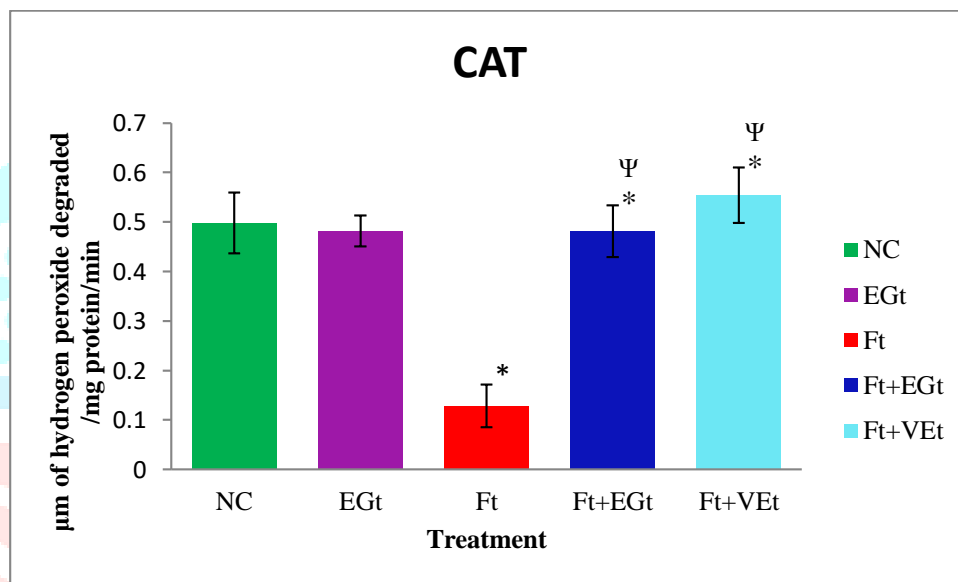


Fig.2. Effect of eugenol on the CAT under fluoride intoxicated condition. The values are significant compared with normal control (\* $p < 0.05$ ).

In the current study, CAT activity in cardiac tissues was significantly increased when sodium fluoride intoxicated rats were given eugenol. Treatment with eugenol for 21 days enhanced the antioxidant enzyme CAT activity of liver in carrageenan-induced arthritis, according to a previous study (Adefegha et al 2018). Rauscher et al (2001) were identified that antioxidant enzyme CAT enhancing capacity of isoeugenol in the tissue such as brain, kidney, heart and liver of streptozotocin induced diabetes Sprague-Dawley rats. As per Oroojan et al (2020), eugenol has an antioxidant impact on oxidative stress that caused by H<sub>2</sub>O<sub>2</sub> in male mice islets of Langerhans via boosting catalytic activity of CAT. Furthermore, eugenol showed antioxidant activity *in vitro* by scavenging hydrogen peroxide produced by the fentons reaction and preventing the development of lipid peroxidation (Nagababu et al 2010). In another *in vitro* free radical scavenging activity assay, eugenol potentially scavenged the hydrogen peroxide on dose dependent manner. Hence it is clear that eugenol has potential hydrogen peroxide scavenging activity in *in vitro* and CAT enhancing capacity in *in vivo*. More importantly, eugenol rich leaf extract of *osimum sanctum* (250 mg/kg) nullified the oxidative stress caused by sodium fluoride in swiss albino mice by increasing CAT enzyme activity (Prakash et al 2018). At the same time, eugenol exhibited the cardio protective role in rats under doxorubicin toxicity (Fouad and Yacoubi, 2011). Gathering all, due to free radical scavenging property and CAT enhancing capacity, eugenol protected the heart tissue under sodium fluoride toxicity induced oxidative stress, in this study.

Further, GPx levels were significantly lower in the Ft group than NC, while GPx levels were significantly higher in Ft+Egt rats when compare to Ft (Fig.3). Treatment of Eugenol to fluoride rats confirmed a greater enhancement in GPx activity relative to fluoride intoxicated group and a slight decrement when compared to standard Vitamin E treatment. When compared to NC, there were no significant changes have been observed in the Egt group.

GPx activity was significantly decreased in the fluoride intoxicated rats, in the present study. The similar results have been observed in Chauhan and colleagues (2013) conducted an experiment, in which female Sprague Dawley rats at the age of 6 and 18 month old were subjected to sodium fluoride. The results revealed that increased oxidative stress reduced glutathione peroxidase activity. In another study, fluoride intoxication for 25 days to adult female rats also found that decreased levels of glutathione reductase activity due to oxidative stress in the liver tissue (Błaszczuk et al 2011). It was also observed that fluoride poisoning reduces glutathione peroxidase enzyme activity in the brain, kidney, liver, plasma, and testis of 8 week old male Wistar rats, according to Inkielewicz-Stepniak and Krechniak (2004). Furthermore, prolonged exposure to sodium fluoride through drinking water for 60 days causes oxidative damage in wistar strain rats' kidney and liver tissues through lowering glutathione peroxidase enzyme activity. Sodium fluoride was also induced the hypertension and oxidative stress causing cardiac damage in heart tissue male albino rats on dose dependent manner (Oyagbemi et al 2016). Hence it is thought that sodium fluoride increased the oxidative stress in different tissues including heart, while in the same manner antioxidant enzyme GPx was decreased in the present study.

In the current research, GPx activity levels were significantly increased in the eugenol treated fluoride intoxicated rats. Similarly, eugenol was decreased the oxidative stress by increasing the glutathione peroxidase activity in liver tissue of rat under arthritis induced by carrageenan (Adefegha et al 2018). A research on the effects of eugenol on glutathione peroxidase in bovine ovarian cortical secondary follicles grown *in vitro* found that eugenol enhanced glutathione peroxidase enzyme activity m-RNA expression levels. Additionally, eugenol prevents cisplatin-induced testicular damage via boosting antioxidant capacity by raising glutathione peroxidase enzyme activity, which was dramatically reduced in the cisplatin-induced rat group (Ekinci Akdemir et al 2019). Moreover, eugenol protected male rats against carbon tetra chloride-induced oxidative stress by boosting glutathione peroxidase enzyme activity, whereas this enzyme activity was dramatically reduced in the carbon tetra chloride toxicity rat group (Kumaravelu et al 1996). In BRL-3A normal rat hepatocytes *in vitro*, another eugenol analogue, aspirin eugenol ester, prevented the paraquat producing oxidative stress caused liver damage by boosting antioxidant enzyme glutathione peroxidase enzyme activity (Zhang et al 2016). The antioxidant enzyme glutathione peroxidase was increased in the Arsenic Trioxide group supplemented with eugenol where it prevented the oxidative stress assisted renal injury in wistar rats, while this enzyme was lowered in the Arsenic Trioxide alone group (Binu et al 2017). Hence, gathering all, it is very clear that eugenol has antioxidant potentiality that correlated with increased antioxidant enzyme glutathione peroxidase enzyme in both previous studies and in the present study.

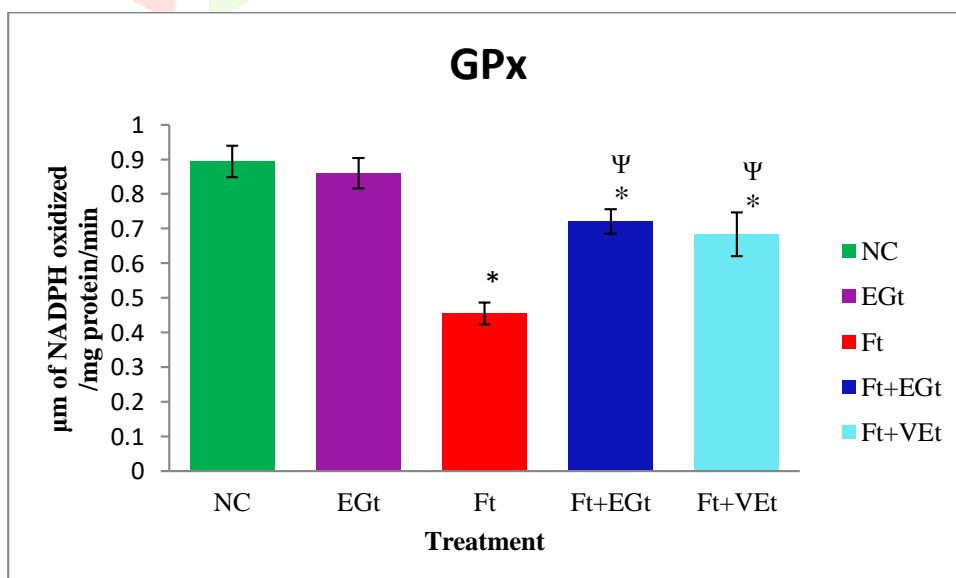


Fig.3. Effect of eugenol on the GPX under fluoride intoxicated condition. The values are significant compared with normal control (\*p<0.05).

Finally, when compared to NC, there was slight GR changes have been observed in the Egt group. While, GR levels were significantly lower in the Ft group than NC, further GR levels were significantly higher in Ft+Egt rats when compare to Ft (Fig.4). Treatment of Eugenol to fluoride rats confirmed a greater enhancement in GR activity relative to fluoride intoxicated group and a slight increment when compared to standard Vitamin E treatment.

The obtained results in the present study were revealed that decreased levels of GR observed in the fluoride intoxicated rat heart tissue. Similar results were observed by Bharti and Srivastava (2009) in the rat brain. They have been administered fluoride orally at a dose of 150 mg/L for 28 days to rats which causing oxidative stress in the brain. Under fluoride-induced oxidative stress, glutathione reductase enzyme levels were shown to be lower. On other hand, Chauhan and colleagues (2013) conducted an experiment, in which female Sprague Dawley rats at the age of 6 and 18 month old were subjected to sodium fluoride. The results revealed that increased oxidative stress reduced glutathione reductase activity. Fluoride consumption of adult female rats for 25 days resulted in reduced glutathione reductase activity owing to oxidative stress in the liver tissue, according to another study (Błaszczuk et al 2011). It was found that sodium fluoride was induced the hypertension and oxidative stress, causing cardiac damage in heart tissue of male albino rats on dose dependent manner (Oyagbemi et al 2016). In addition, female wistar strain rats were given 150 ppm sodium fluoride in their drinking water. Sodium fluoride caused oxidative stress in numerous tissues such the heart, liver, and kidneys by inhibiting antioxidant enzyme like glutathione reductase (Bharti et al 2014). Fluoride has a detrimental influence on the antioxidant system through changing antioxidant enzymes such as glutathione reductase, as evidenced by all prior findings. It also validates the findings of the current study, which show that fluoride inhibits the glutathione reductase enzyme in heart tissue.

Increased glutathione reductase activity was observed in the fluoride intoxicated rats supplemented with eugenol natural compound. It was found that eugenol has the antioxidant activity that reduces the oxidative stress under illness. In a study, eugenol protected male rats against carbon tetra chloride-induced oxidative stress by boosting glutathione reductase enzyme activity, whereas this enzyme activity was dramatically reduced in the carbon tetra chloride toxicity rat group (Kumaravelu et al 1996). In a study, sub-acute administration of antioxidant compound isoeugenol in 30-day streptozotocin-induced diabetic Sprague-Dawley strain rats was studied by Rauscher and his colleagues in the year 2001. Eugenol enhanced glutathione reductase activity in the hepatic, renal, brain, and cardiac tissues, but this enzyme activity was lowered in the organs examined. Eugenol enhanced antioxidant capacity by raising glutathione reductase activity levels in concanavalin-A assisted T-cell-mediated acute hepatitis, but its levels were lowered in the hepatitis state. It was also found that eugenol rich extract of clove significantly decreased the oxidative stress in liver cirrhosis by increasing glutathione reductase activity, while glutathione reductase activity was significantly decreased in liver cirrhosis (Ali et al 2014).

Glutathione reductase activity involves conversion of oxidized glutathione into reduced form. While, reduced glutathione is very essential for the glutathione peroxidase enzyme. By utilizing reduced glutathione, glutathione peroxidase enzyme converts hydrogen peroxide into water, where hydrogen peroxide is very toxic to the cell. At the same time increased levels of glutathione peroxidase enzyme in the eugenol treated fluoride intoxicated rats, in the present study also support the increased levels of glutathione reductase activity. It is very clear the both glutathione peroxidase and glutathione reductase activities are interdependent. Hence, it is very clear that eugenol has antioxidant property due to its role in increasing levels of antioxidant enzymes such as glutathione reductase and glutathione peroxidase.

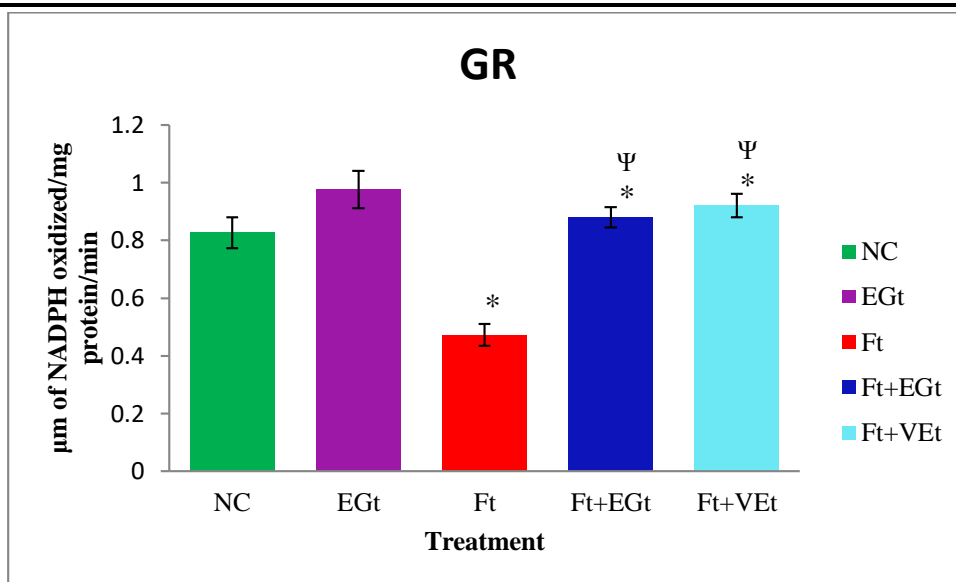


Fig.4. Effect of eugenol on the GR under fluoride intoxicated condition. The values are significant compared with normal control (\* $p < 0.05$ ).

## CONCLUSION

The findings of this study reveal that the eugenol can protect cardiac tissue under oxidative stress caused by fluoride by enhancing antioxidant enzyme. In future, clinical studies on the action of eugenol must be determined in fluoride intoxicated people.

## ACKNOWLEDGEMENT

The corresponding author expresses sincere gratitude to the University Grants Commission, New Delhi, India for the financial assistance through UGC-BSR Faculty Fellowship (No: F.18-1/2011 (BSR), Dated 24-11-2017) and Major Research Project UGC MR-Major-Zol 2013-36027 dated 15/10/2015.

## CONFLICT OF INTEREST

All authors declare that there is no conflict of interest.

## REFERENCES

1. Amini H, Taghavi Shahri SM, Amini M, Ramezani Mehrian M, Mokhayeri Y, Yunesian M. Drinking water fluoride and blood pressure? An environmental study. *Biol Trace Elem Res.* 2011; 144(1-3): 157-63. doi: 10.1007/s12011-011-9054-5.
2. Varol E, Akcay S, Ersoy IH, Koroglu BK, Varol S. Impact of chronic fluorosis on left ventricular diastolic and global functions. *Sci Total Environ.* 2010; 408(11): 2295-8. doi: 10.1016/j.scitotenv.2010.02.011.
3. Kiyuna LA, Albuquerque RPE, Chen CH, Mochly-Rosen D, Ferreira JCB. Targeting mitochondrial dysfunction and oxidative stress in heart failure: Challenges and opportunities. *Free Radic Biol Med.* 2018; 129: 155-168. doi:10.1016/j.freeradbiomed.2018.09.019.
4. Chattopadhyay, A., Podder, S., Agarwal, S. et al. Fluoride-induced histopathology and synthesis of stress protein in liver and kidney of mice. *Arch Toxicol.* 2011; 85: 327–335. doi.org/10.1007/s00204-010-0588-7.
5. Kollmannsberger H, Nitz S. *Technol. Lebensmitt.* 1994; 16(3/4): 112–123.
6. Teresa M, Trevison S, Goretti M, Silva V, Pfundstein B, Spiegelhalter B, Owen RW. Characterization of the volatile pattern and antioxidant capacity of essential oils from different species of the genus *Ocimum*. *J. Agric. Food Chem.* 2006; 54: 4378-4382.
7. Binu P, Priya N, Abhilash S, Vineetha RC, Nair RH. Studies on curative efficacy of monoterpene eugenol on anti-leukemic drug arsenic trioxide induced cardiotoxicity. *Biomedicine & pharmacotherapy.* 2017; 91: 559-566. doi.org/10.1016/j.biopha.2017.04.087.
8. Choudhary R, Mishra KP, Subramanyam C. Interrelations between oxidative stress and calcineurin in the attenuation of cardiac apoptosis by eugenol. *Molecular and cellular biochemistry.* 2006; 283(1-2): 115-122. doi.org/10.1007/s11010-006-2386-3.



9. Ito M, Murakami K, Yoshino M. Antioxidant action of eugenol compounds: role of metal ion in the inhibition of lipid peroxidation. *Food and chemical toxicology: an international journal published for the British Industrial Biological Research Association*. 2005; 43(3): 461-466. doi.org/10.1016/j.fct.2004.11.019.
10. Kelm MA, Nair MG, Strasburg GM, DeWitt DL. Antioxidant and cyclooxygenase inhibitory phenolic compounds from *Ocimum sanctum* Linn. *Phytomedicine*. 2000; 7(1): 7-13. doi:10.1016/s0944-7113(00)80015-x.
11. Jang IS, Chae KR, Cho JS. Effects of age and strain on small intestinal and hepatic antioxidant defense enzymes in Wistar and Fisher 344 rats. 2001; 122(6): 561-570. doi.org/10.1016/S0047-6374(01)00236-6.
12. Misra HP, Fridovich I. The role of superoxide anion in the autooxidation of epinephrine and a simple assay for superoxide dismutase. *K. Biol Chem*. 1972; 247: 3170-3175.
13. Aebi H. Catalase in vitro. *Methods Enzymol*. 1984; 105: 125-126.
14. Flohe L, Gunzler WA. Glutathione Peroxidase. *Methods Enzymol*. 1984; 105: 115-121.
15. Carlberg I, Mannervik B. Glutathione reduction. In: *Methods in Enzymology* ed., Colowick, S.P. and Kaplan, N.O. Academic Press in Orlando. 1985; 113: 484-499.
16. Kangralkar VA, Patil SD, Bandivadekar RM. Oxidative stress and diabetes: A review. *Intl J Pharm Appl*. 2010; 1: 38-45.
17. Yasui K, Baba A. Review Therapeutic potential of superoxide dismutase (SOD) for resolution of inflammation. *Inflamm Res*. 2006; 55(9): 359-363.
18. Farías JG, Molina VM, Carrasco RA, et al. Antioxidant Therapeutic Strategies for Cardiovascular Conditions Associated with Oxidative Stress. *Nutrients*. 2017; 9(9): 966. doi:10.3390/nu9090966.
19. Sánchez-Villamil JP, D'Annunzio V, Finocchietto P, Holod S, Rebagliati I, Pérez H, Peralta JG, Gelpi RJ, Poderoso JJ, Carreras MC. Cardiac-specific overexpression of thioredoxin 1 attenuates mitochondrial and myocardial dysfunction in septic mice. *Int. J. Biochem. Cell Biol*. 2016; 81: 323-334. doi:10.1016/j.biocel.2016.08.045.
20. Rathore KI, Kerr BJ, Redensek A, López-Vales R, Jeong SY, Ponka P, David S. Ceruloplasmin protects injured spinal cord from iron-mediated oxidative damage. *J. Neurosci*. 2008; 28: 12736-12747. doi: 10.1523/JNEUROSCI.3649-08.2008.
21. Zhan XA, Wang M, Xu ZR, Li WF, Li JX. Effects of fluoride on hepatic antioxidant system and transcription of Cu/Zn SOD gene in young pigs. *J Trace Elem Med Biol*. 2006; 20(2): 83-87. doi: 10.1016/j.jtemb.2005.11.003.
22. Sun Z, Li S, Guo Z, Li R, Wang J, Niu R, Wang J. Effects of Fluoride on SOD and CAT in Testis and Epididymis of Mice. *Biol Trace Elem Res*. 2018; 184(1): 148-153. doi: 10.1007/s12011-017-1181-1.
23. Basha MP, Sujitha NS. Chronic fluoride toxicity and myocardial damage: antioxidant offered protection in second generation rats. *Toxicol Int*. 2011; 18(2): 99-104. doi:10.4103/0971-6580.84260.
24. Nagababu E, Rifkind JM, Boindala S, Nakka L. Assessment of antioxidant activity of eugenol in vitro and in vivo. *Methods Mol Biol*. 2010; 610: 165-80. doi: 10.1007/978-1-60327-029-8\_10.
25. Ekinci Akdemir FN, Yildirim S, Kandemir FM, Aksu EH, Guler MC, Kiziltunc Ozmen H, Kucukler S, Eser G. The antiapoptotic and antioxidant effects of eugenol against cisplatin-induced testicular damage in the experimental model. *Andrologia*. 2019; 51(9): e13353. doi: 10.1111/and.13353.
26. Kumaravelu P, Subramaniyam S, Deepalakshmi P, Dakshinamoorthy N, Devaraj S. The antioxidant effect of eugenol on CCl4-induced erythrocyte damage in rats. *The Journal of Nutritional Biochemistry*. 1996; 7(1): 23-28.
27. Rizvi S, Raza ST, Ahmed F, Ahmad A, Abbas S, Mahdi F. The role of vitamin e in human health and some diseases. *Sultan Qaboos Univ Med J*. 2014; 14(2):e157-e165.
28. Schonbaum GR, Chance B. in: *The Enzymes* (P. D. Boyer, ed.), Academic Press, New York. 1976; 13: 363-409.
29. Stein K, Hain JU. Catalase biosensor for the determination of hydrogen peroxide, fluoride and cyanide. *Mikrochim Acta*. 1995. 118: 93-101. doi.org/10.1007/ BF01242232.
30. Cicek E, Aydin G, Akdogan M, Okutan H. Effects of chronic ingestion of sodium fluoride on myocardium in a second generation of rats. *Hum Exp Toxicol*. 2005; 24(2): 79-87. doi: 10.1191/0960327105ht505oa.
31. Adefegha SA, Oyeleye SI, Okeke BM, Oboh G. Influence of eugenol on oxidative stress biomarkers in the liver of carrageenan-induced arthritis rats. *J Basic Clin Physiol Pharmacol*. 2018; 30(2): 185-193. doi: 10.1515/jbcpp-2018-0060.

32. Rauscher FM, Sanders RA, Watkins JB. Effects of isoeugenol on oxidative stress pathways in normal and streptozotocin-induced diabetic rats. *J Biochem Mol Toxicol*. 2001; 15(3): 159-164. doi: 10.1002/jbt.13.
33. Oroojan AA, Chenani N, An'aam M. Antioxidant Effects of Eugenol on Oxidative Stress Induced by Hydrogen Peroxide in Islets of Langerhans Isolated from Male Mouse. *Int J Hepatol*. 2020; 2020: 5890378. doi: 10.1155/2020/5890378.
34. Prakash B, Suresh Kumar S, Rajbala V, John PJ, Inderpal, Soni. Sodium Fluoride-Induced Oxidative Stress and Histological Changes in Liver of Swiss Albino Mice and Amelioration by *Ocimum Sanctum* Linn. *Asian J Pharm Clin Res*. 2018; 11(9): 195-199.
35. Fouad AA, Yacoubi MT. = Mechanisms underlying the protective effect of eugenol in rats with acute doxorubicin cardiotoxicity. *Arch Pharm Res*. 2011; 34(5): 821-828. doi: 10.1007/s12272-011-0516-2.
36. Chauhan SS, Mahmood A, Ojha S. Ethanol and age enhance fluoride toxicity through oxidative stress and mitochondrial dysfunctions in rat intestine. *Mol Cell Biochem*. 2013; 384(1-2): 251-262. doi: 10.1007/s11010-013-1804-6.
37. Błaszczuk I, Birkner E, Kasperczyk S. Influence of methionine on toxicity of fluoride in the liver of rats. *Biol Trace Elem Res*. 2011; 139(3): 325-331. doi: 10.1007/s12011-010-8664-7.
38. Inkielewicz I, Krechniak J. Fluoride effects on glutathione peroxidase and lipid peroxidation in rats. *Fluoride*. 2004; 37(1): 7-12.
39. Oyagbemi AA, Omobowale TO, Asenuga ER, Adejumobi AO, Ajibade TO, Ige TM, Ogunpolu BS, Adedapo AA, Yakubu MA. Sodium fluoride induces hypertension and cardiac complications through generation of reactive oxygen species and activation of nuclear factor kappa beta. *Environ Toxicol*. 2017; 32(4): 1089-1101. doi: 10.1002/tox.22306.
40. Zhang S, Niu Q, Gao H, Ma R, Lei R, Zhang C, Xia T, Li P, Xu C, Wang C, Chen J, Dong L, Zhao Q, Wang A. Excessive apoptosis and defective autophagy contribute to developmental testicular toxicity induced by fluoride. *Environmental pollution*. 2016; 212: 97-104. doi.org/10.1016/j.envpol.2016.01.059.
41. Bharti VK, Srivastava RS. Fluoride-induced oxidative stress in rat's brain and its amelioration by buffalo (*Bubalus bubalis*) pineal proteins and melatonin. *Biol Trace Elem Res*. 2009; 130(2): 131-140. doi: 10.1007/s12011-009-8320-2.
42. Bharti VK, Srivastava RS, Kumar S, Bag AC, Majumdar G, Singh SR, Perumal P, Gregory M, Brown. Effects of Melatonin and Epiphyseal Proteins on Fluoride-Induced Adverse Changes in Antioxidant Status of Heart, Liver, and Kidney of Rats. *Advances in Pharmacological and Pharmaceutical Sciences*. 2014; 6. doi.org/10.1155/2014/532969.
43. Ali AO, Narges C, Marzieh A. Antioxidant Effects of Eugenol on Oxidative Stress Induced by Hydrogen Peroxide in Islets of Langerhans Isolated from Male Mouse. *International Journal of Hepatology*. 2020; Article ID 5890378, 5. doi.org/10.1155/2020/5890378.