Research Article **ISSN: 2349 – 7106**

Asian Journal of Research in Chemistry and Pharmaceutical Sciences

Journal home page: www.ajrcps.com

https://doi.org/10.36673/AJRCPS.2021.v09.i03.A20

EFFECT OF FERULIC ACID ON MYOCARDIAL TISSUE LEVELS OF TBARS, GSH, SOD, CATALASE AND TISSUE NITRATE IN RATS

K. Sowmya*[1], B. Navya[1], G. Spandana[1], B. Sushma Sagri[1]

[1*]Srinivasa Rao College of Pharmacy, Pothina Mallayya Palem, Madurawada, Vishakapatna, Andhra Pradesh, India.

ABSTRACT

The present study was designed to evaluate the cardioprotective role of Ferulic acid on the *in vivo* myocardial ischemic reperfusion injury in rats. **Methods:** Male Wistar albino rats were divided into six groups (n=6). The group C and C-IR animals were administered saline orally (sham, I-R Control group), animals group FA-BL1 and FA-BL2 received ferulic acid 20mg/kg and 40mg/kg b.wt respectively upto 30 days orally without inducing I-R injury and animals group. FA-IR1 and FA-IR2 received FA 20mg/kg and 40mg/kg respectively upto 30 days. On the 30th day animals of C-IR, FA-IR1 and FA-IR2 groups were underwent 15 minutes of ligation of left anterior descending coronary artery and were thereafter reperfused by 60 minutes. **Results:** In the C- IR group, myocardial infarct size was observed by the presence of unstained necrotic tissue, a significant decrease in the levels of endogenous anti oxidant enzymes (GSH, CAT, SOD), tissue nitrate (NO), cardiac markers (LDH, CK, AST) and increased the lipid peroxidation levels (TBARS) due to the myocardial damage by ischemic reperfusion injury. Chronic oral administration of FA at both doses (20mg/kg and 40mg/kg) significantly restored the myocardial anti oxidant status evidenced by increased SOD, CAT and GSH, tissue nitrate levels, diminished the TBARS levels and prevented the leakage of cardio-specific enzymes LDH, CK, AST. This is further confirmed by histopatholoical changes. **Conclusion:** Ferulic acid significantly attenuates myocardial ischemic reperfusion injury through augmentation of endogenous antioxidants, stabilization of myocardial membrane permeability and induction of nitric oxide production.

KEYWORDS

Ferulic acid, TBARS levels, CAT and GSH.

Author for Correspondence:
Sowmya K,
Srinivasa Rao College of Pharmacy,
Vishakapatna, Andhra Pradesh, India.

Email: gerapatikiran@gmail.com

INTRODUCTON

Cardiovascular disease

Cardiovascular diseases (CVDs) are a group of disorders of the heart and blood vessels and they include: coronary heart disease (CHD), stroke, peripheral vascular disease, congenital heart disease, endocarditis, heart failure.

Coronary heart disease – disease of the blood vessels supplying the heart muscle;
Cerebrovascular disease - disease of the blood vessels supplying the brain;
Peripheral arterial disease – disease of blood vessels supplying the arms and legs;
Rheumatic heart disease – damage to the heart muscle and heart valves from rheumatic fever, caused by streptococcal bacteria;
Congenital heart disease - malformations of heart structure existing at birth;
Deep vein thrombosis and pulmonary embolism – blood clots in the leg veins, which can dislodge and move to the heart and lungs.
Heart attacks and strokes are usually acute events and are mainly caused by a blockage that prevents blood from flowing to the heart or brain. An ischemic stroke (the most common type) happens when a blood vessel that feeds the brain gets blocked, usually from a blood clot. When the blood supply to a part of the brain is shut off, brain cells will die. The result will be the inability to carry out some of the previous functions as before like walking or talking. A hemorrhagic occurs when a blood vessel within the brain bursts. The most likely cause was uncontrolled hypertension.

Animals

A total of 30 male albino mature rats, weighing 150 to 200g, were used in the study. Animals were obtained from the animal house of In-vivo life sciences, Bangalore, for experimental purpose. Rats were housed in polypropylene cages with not more than four animals per cage in an air conditioned room and allowed access to pellet diet (Sai Durga Feeds and Foods, Bangalore) and water *ad libitum*. The animals were maintained under controlled conditions of temperature (23±2^{o}C), humidity (50±5%) and 12 h light – dark cycles. Anima
ls were habituated to laboratory conditions for 48 h prior to experimental protocol to minimize if any of non-specific stress. The study protocol was reviewed and approved by the Institutional animal CPCSEA, New Delhi (India) and the Institutional Animal Ethics Committee (1220/a08/CPCSEA) approved the experimental protocol.

Experimental protocol

Animals were divided into six experimental groups comprising six animals each.
Haemodynamically unstable rats were excluded from the study.

Group-C

Designated as Sham control received normal saline (0.9%) once daily for 30 days orally and then sacrificied on 30th day. Rats underwent the entire surgical procedure except the LAD occlusion.

Group C-IR

Received normal saline (0.9%) once daily for 30 days orally and in addition, underwent LAD coronary artery ligation for 15minutes followed by 60 minutes of reperfusion.

Group FA-BL1

Received FA (20mg/kg b.w) once daily for 30 days orally. On the 30th day, rats underwent the entire surgical procedure except LAD occlusion.

Group FA-BL2

Received FA (40mg/kg b.w) once daily for 30 days orally. On the 30th day, rats underwent the entire surgical procedure except LAD occlusion.

Group FA-IR1

Received FA (20mg/kg b.w) once daily for 30 days orally. On the 30th day, rats underwent LAD coronary artery ligation for 15minutes followed by 60 minutes of reperfusion.

Group FA-IR2

Received FA (40mg/kg b.w) once daily for 30 days orally. On the 30th day, rats underwent LAD coronary artery ligation for 15minutes followed by 60 minutes of reperfusion.

Induction of *in vivo* myocardial ischemic reperfusion

All the animals anesthetized intramuscularly with phenobarbitone (35mg/kg b.w.) and ketamine (20mg/kg b.w.). The body temperature was monitored and maintained at 37°C throughout the experimental protocol. Neck was opened and tracheostomy was performed for the ventilation of animal. A left anterior descending coronary artery was examined and verified visually and then ligated 4-5mm from its origin. A 6.0 suture was placed around the LAD coronary artery, midway between

the apex of the heart and the left atrial appendage for ligation. The thoracic cavity was covered with saline soaked gauze after the surgery to prevent the heart from drying. After completion of surgical procedure, heart was returned to its normal position in thorax. The animals then underwent 15minutes of ischemia and reperfused by releasing snare gently for a period of 60 min. At the end of the experiment, animals were sacrificed by a over dose of anaesthesia and heart were excised. Heart was washed with chilled phosphate buffer saline solution (P^H-7.4) and subjected for biochemical parameters, myocardial infract area and histopathological studies.

Preparation of tissue homogenate

About 500mg of heart tissue was homogenized in 5ml of phosphate buffer (P^H 7.4) the homogenate was centrifuged at 7000rpm for 15min and the supernatant was used for biochemical parameters.

Biochemical parameters like

Thio barbituric acid reactivating substances (TBARS)

Reduced glutathione (GSH)

Superoxide dismutase (SOD)

Estimation of biochemical parameters

Estimation of TBARS levels

TBARS levels were determined by a modified version of the method described by Ohkawa.[115]

Principle

Acetic acid was used to detach the lipid and protein of the tissue and the protein in the reaction mixture were dissolved by the addition of sodium lauryl sulphate. Thiobarbituric acid reacts with lipid peroxides, hydroperoxide and oxygen double bond to form a coloured adduct with absorption maximum at 532nm, which was then measured.

Reagents

Sodium lauryl sulphate - 8.1% (W/V) Acetic acid - 20% (P^H – 3.5) Thiobarbituric acid - 0.8% (w/v) Butanol: Pyridine - (15:1) (v/v) Trichloracetic acid (TCA) - 10%

Procedure

Tissue was homogenized with 10% Trichloroacetic acid (TCA) in 1:10 ratio (for 1gm of tissue 10ml of 10% TCA was added) and centrifuged at 3000 × g for 10 min. 0.2ml of whole homogenate was taken to which 0.2ml of 8.1% sodium lauryl sulfate, 1.5ml of 20% acetic acid and 1.5ml 0.8% thiobarbituric acid were added. Volume was made up to 4ml with double distilled water. It was heated at 95°C for 60 min. After cooling, 1ml of double distilled H_2O and 5ml of butanol-pyridine mixture was added. The solution was shaken vigorously in a vortex and centrifuged at 4000rpm for 10 min in a cold centrifuge. The organic layer was separated and absorbance was observed at 532nm in a spectrophotometer.

Standard curve

Various concentrations of 1, 1, 3, 3-Tetra methoxy propane (TMP) were used as external standard (1-10nm) and were subjected to the steps mentioned in the procedure section. The readings of absorbance were plotted against the concentration of TMP to derive a linear standard graph. Data expressed as nmole/g wet wt. tissue

Calculation

The concentration of TBARS was determined from the linear standard graph.

Estimation of GSH levels

Glutathione was estimated by the method described by Ellman[116].

Principle

Bis (p-nitro phenyl) disulphide reacts with aliphatic thiol compounds at pH 8.0 to produce one mole of p-nitrothiophenol anion per mole thiol. Since this anion is highly coloured ($\lambda m \approx 13$, 6000 at 412nm), it can be used to measure the thiol concentration.

Reagents

Phosphate buffer (K2HPO4) - 0.3 M (P^H-8.4)

5, 5` - Dithiobis (2- nitrobenzoic acid) - 0.2% w/v (DTNB) Sulfosalicyclic acid - 10% v/v

Procedure

The hearts were homogenized with 10% TCA in 1:10 ratio (for 1gm of tissue 10ml of 10% TCA was added) and centrifuged at 3000 × g for 10 min. The reaction mixture contained 0.1 ml of supernatant, 2.0ml of 0.3 M phosphate buffer (pH-8.4), 0.4ml of double distilled water and 0.5ml of DTNB [5, 5'dithiobis (2-nitrobenzoic acid)]. The reaction mixture was incubated for 10 min and the absorbance was measured at 412 nm in a spectrophotometer.

Standard Curve

Various concentrations of the standard glutathione (1-10µg) were subjected to the steps mentioned above. The readings of absorbance were plotted against the concentration of GSH to produce a linear standard graph.

Calculation

The concentration of GSH was determined from the linear standard graph. Data are expressed as µmole per gm wet weight.

Estimation of SOD levels

SOD levels in the hearts were determined by the method of McCord and Firdovich method (1969) and modified by Kakkar[117].

Principle

Superoxide anions were generated in a system comprising of NADH and phenazine methosulphate. This superoxide anion reduced into blue tetrazolium forming a blue Formozan, which was measured at 560 nm. SOD inhibited the reduction of nitro blue tetrazolium and thus the enzyme activity was measured by monitoring the rate of decrease in optical density at 560nm.

Reagents

Sodium pyrophosphate - 0.052M, P^H 8.3

Phenazinemethosulphate - 186mM

Nitrobluetetrazolium - 300mM.

Glacial acetic acid - 60.05M Tris buffer with sucrose - 0.25M

Tris HCL buffer - 0.0025M, P^H 7.4

Procedure

The tissue was homogenized in 10% TCA in 1:10 ratio (for 1gm of tissue 10ml of 10%TCA was added) and centrifuged at 3000 × g for 10 min. Then the homogenized tissue was centrifuged at 10,000rpm for 15 min at 4°C. The supernatant was collected, to which 50% ammonium sulphate was added, vortexed, and the reaction mixture was kept for incubation at 4°C for 4 h. After the incubation period it was again centrifuged at 12,000 rpm for 30 min at 4°C. The samples were then kept overnight for dialysis in 0.0025 Tris HCl buffer. The next day, appropriate volumes of samples to a maximum of

1.2ml were taken. To it, 1.2ml sodium pyrophosphate, 0.1ml of phenazine methosulphate, 0.3ml of NBT and 0.2ml NADH were added. The final volume was made up to 3 ml with distilled water. After adding NADH, it was immediately incubated for 90 sec at 30°C and the reaction was stopped by adding 1ml acetic acid to the reaction mixture. 4ml of butanol was added and after 10min, centrifuged at 3000rpm, for 10 min. The organic layer was separated and absorbance was observed at 560nm in aspectrophotometer.

Standard curve

Various concentrations of standard SOD (0.1 to 2.0 µg) were subjected to the same steps as the samples mentioned in the above procedure. The reading of absorbance was plotted against the concentrations of SOD to derive a linear standard graph.

Calculation

The concentration of SOD was determined from the linear standard graph. Data expressed as IU/mg protein.

Determination of myocardial infarct size

At the end of this *in-vivo* study, the heart was rapidly excised and retrogradely perfused with ice cold saline solution to remove any blood and injected the 2% 0.25ml Triphenyl tetrazolium chloride. The heart was sectioned transversely from the apex to the base in five slices of 2-mm thickness, and incubated in phosphate buffer (pH 7.4, 37°C) for 12 hrs to stain viable tissue red and necrotic tissue appears as pale/grey colour.

Histopathology studies

Light microscopy

The hearts were removed, washed immediately with saline and then fixed in 10% buffered formalin.

Statistical analysis

Values are expressed as mean ± SEM and analyzed using Graph Pad prism version No.1 using ANOVA fallowed by Tukey multiple comparision test. $P<0.05$ was considered significantly.

RESULTS AND DISCUSSION

Effect of ferulic acid on tbars levels

There is significantly ($p<0.0001$) increase in the level of T-BARS in C-IR Group (10.06±0.63nmol/g) when compared with control group (C) (2.5±0.16nmol/g). No significance difference in the FA-BL1 (3.5±0.08nmol/g) and

FA-BL2 (3.4±0.43nmol/g) when compared to control group. Significant decrease ($p<0.0001$) in the level of TBARS in FA-IR1 (6±0.84nmol/g) and FA-IR2 (6.9±0.23nmol/g) when compared to C-IR group. The Results were noted in Table No.1 and Graph No.1.

Effect of ferulic acid on GSH levels

There is significantly ($p<0.0001$) decrease in the level of Glutathione in C-IR Group (50.02±1.5µg/g) when compared with control group (C) (80.9±3.3 µg/g). No significance difference in the FA-BL1 (84.4±2.1µg/g) and FA-BL2 (85.6±1.8 µg/g) when compared to control group. Significant increase ($p<0.0001$) in the level of GSH in FA-IR1 (74.5±0.23µg/g) and FA-IR2 (101±7.9µg/g) when compared to C-IR group. The Results were shown in Table No.1 and Graph No.2.

Effect of ferulic acid on SOD levels

There is significantly ($p<0.001$) decrease in the level of SOD in C-IR Group (0.45±0.13IU/dl) when compared with control group (C) (5.3±0.38 IU/dl). No significance difference in the FA-BL1 (4.2±0.26 IU/dl) and FA-BL2 (7±0.26 IU/dl) when compared to control group. Significant increase ($p<0.0001$) in the level of SOD in FA- IR1 (7.3±0.55 IU/dl) and FA-IR2 (9.3±1.6 IU/dl) when compared to C-IR group. The Results were shown in Table No.1 and Graph No.3.

Effect of ferulic acid on myocardial infarction area

Effect of Ferulic acid against IR damage in rats evaluated by 2, 3, 5 - triphenyltetrazolium chloride (TTC) staining. Group C shows normal myocardium. In C- IR group infracted area was identified by the presence of scattered patches of necrotic tissue were clearly visible as the unstained infracted region. Animals treated with ferulic acid (20 and 40mg/kg) were showed markedly reduced infarction in heart tissue. The results were shown in Figure No.1.

Discussion

The present study showed that pre-treatment with Ferulic acid reduced ischemic-reperfusion injury in rat heart by improving endogenous antioxidants (GSH, SOD and Catalase), inhibition of lipid peroxidation (TBARS) and also by inhibition of leakage of cardiac marker enzymes (LDH, CK and AST) and protected myocardial histoarchitecture.

In the present study ischemic reperfusion injury associated with oxidative stress, as evidenced by increased myocardial TBARS level as oxidative stress marker and depletion of myocardial endogenous antioxidant system in C-IR group.

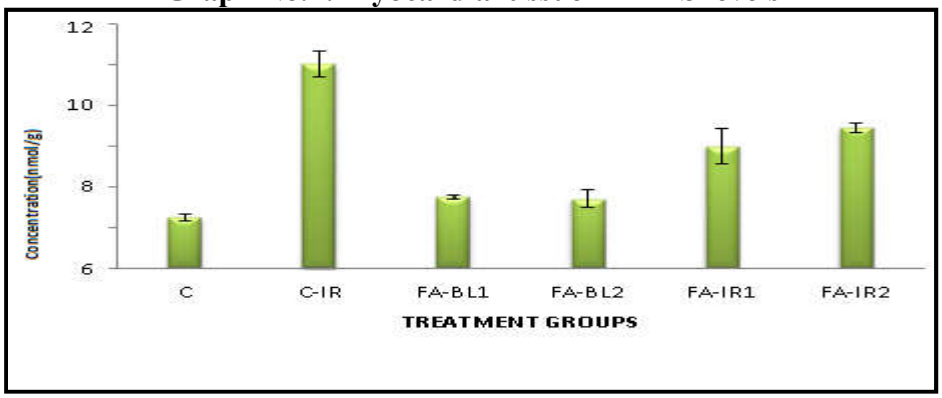
In the present study we observed that the oral chronic administration of ferulic acid at two different doses significantly decreases the myocardial TBARS and concomitant increase in the levels of endogenous antioxidants (GSH, SOD and catalase). Elevated levels of myocardial TBARS is an indicative for increase in the oxidative stress during ischemia which might be stimulated the self protective mechanism via increasing in the level of myocardial endogenous antioxidant systems.

Table No.1: Effect of ferulic acid on tbars, GSH, SOD levels

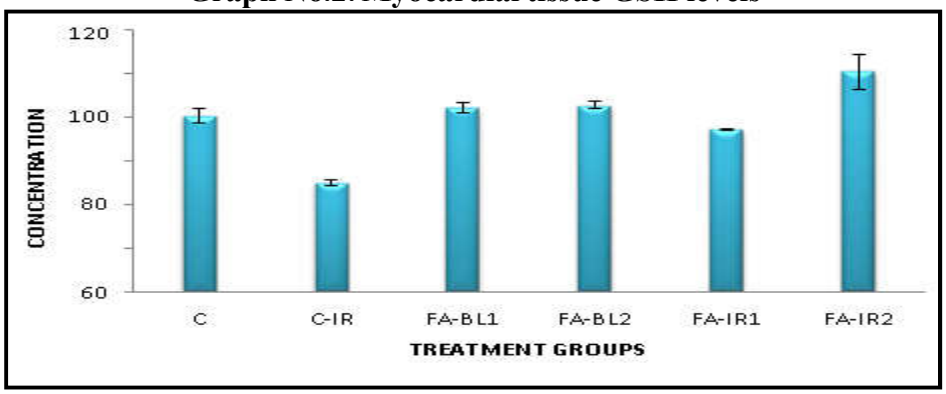
S.No	Treatment roups	TBARS (nmol/g)	GSH (µg/g)	SOD(IU/dl)	Catalase (IU/dl)	Tissue Nitrate (µg/dl)
1	C	2.5±0.16	80.9±3.3	5.3±0.38	102.4±0.98	1.6±0.19
2	C-IR	10.06±0.63***	50.02±1.5***	0.45±0.13**	30.51±1.1***	1.1±0.05**
3	FA-BL1	3.5±0.08	84.4±2.1	4.2±0.26	91.4±0.84	1.4±0.04
4	FA-BL2	3.4±0.43	85.6±1.8	7±0.26	84.8±2.8	2.2±0.03
5	FA-IR1	6±0.84***	74.5±0.23***	7.3±0.55***	42.8±1.3***	1.8±0.06***
6	FA-IR2	6.9±0.23***	101±7.9***	9.3±1.6***	76.8±2.6***	2.2±0.14***

All values expressed as mean±SEM. One-way analysis of variance followed by Tukey's Multiple Comparison Test. ** P<0.001; *** P<0.0001.

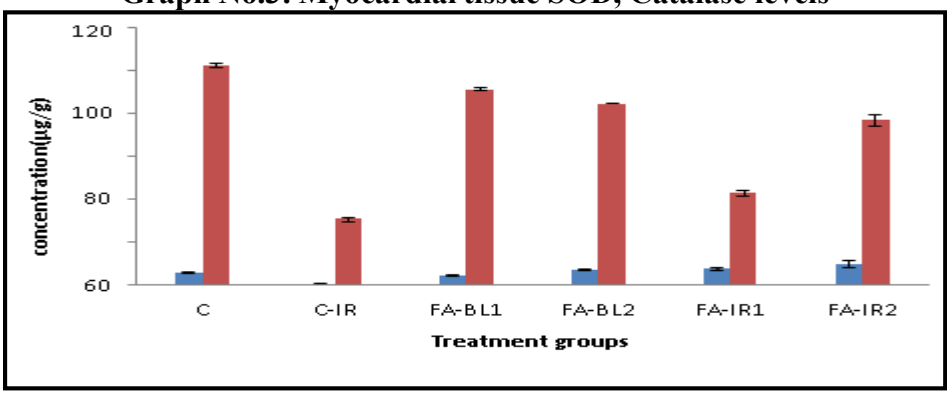
Graph No.1: Myocardial tissue TBARS levels



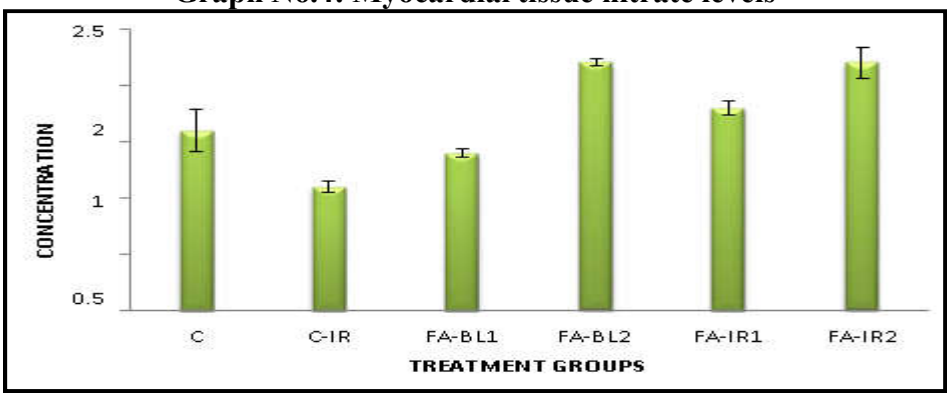
Graph No.2: Myocardial tissue GSH levels



Graph No.3: Myocardial tissue SOD, Catalase levels



Graph No.4: Myocardial tissue nitrate levels



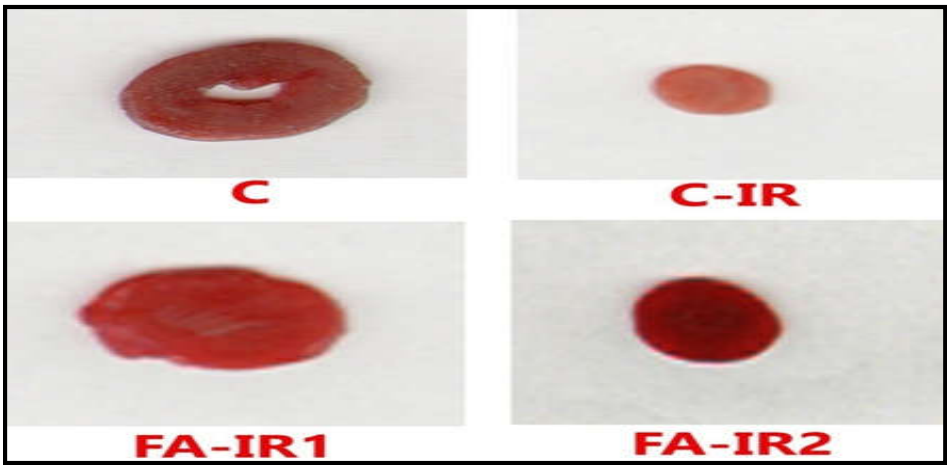


Figure No.1: Effect of Ferulic acid on myocardial infarct size

CONCLUSION

The present study provides unequivocal evidence that the ferulic acid significantly attenuates myocardial ischemic reperfusion injury, and the effect is accomplished through augmentation of endogenous antioxidants, stabilization of myocardial membrane permeability and induction of nitric oxide production.

ACKNOWLEDGEMENT

The authors wish to express their sincere gratitude to Srinivasa Rao College of Pharmacy, Pothina Mallayya Palem, Madurawada, Vishakapatna, Andhra Pradesh, India for providing necessary facilities to carry out this research work.

CONFLICT OF INTEREST

We declare that we have no conflict of interest.

BIBLIOGRAPHY

1. Global status report on noncommunicable disaeses 2010, *World Health Organization, Geneva,* 2011.
2. Atlas of Heart Disease and stroke, *WHO,* 2004.
3. British Heart Foundation, *European Cardiovascular Disease Statistics,* 2000.
4. WHO. World Health Report, 2002.
5. British Heart Foundation, *Coronary Heart Disease Statistics,* 2004.
6. Lim S S, Vos T, Flaxman A D, Danaei G, Shibuya K, Adair-Rohani H *et al*. A comparative risk assessment of burden of disease and injury attributable to 67 risk factors and risk factor clusters in 21 regions, 1990-2010: A systematic analysis for the Global Burden of Disease Study 2010, *Lancet,* 380(9859), 2012, 2224-2260.
7. The global burden of disease: 2004 update, *World Health Organization, Geneva,* 2008.
8. World Heart Federation Fact-Sheet, 2002.
9. Global atlas on cardiovascular disease prevention and control, *World Health Organization, Geneva,* 2011, 155.
10. Mathers C D, Loncar D. Projections of global mortality and burden of disease from 2002 to 2030, *PLoS Med,* 3(11), 2006, e442.
11. Divya pamnani. A new look at the cardiovascular crisis in India, *www.expresspharmaonline.com,* 2009.
12. Dinesh C. Sharma. India's no.1 killer: Heart disease, *India today.in,* 2010.

Please cite this article in press as: Sowmya K *et al*. Effect of ferulic acid on myocardial tissue levels of Tbars, GSH, SOD, catalase and tissue nitrate in rats, *Asian Journal of Research in Chemistry and Pharmaceutical Sciences,* 9(3), 2021, 148-154.