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Original article:

PROTECTIVE EFFECT OF *TRIBULUS TERRESTRIS* L. FRUIT AQUEOUS EXTRACT ON LIPID PROFILE AND OXIDATIVE STRESS IN ISOPROTERENOL INDUCED MYOCARDIAL NECROSIS IN MALE ALBINO WISTAR RATS

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ABSTRACT

The objective of the present study was to evaluate the possible protective effects of *Tribulus terrestris fruit aqueous extract* (TTFAEt) on lipid profile and oxidative stress in isoproterenol (ISO) induced myocardial necrosis in albino Wistar rats. Albino Wistar rats were divided into normal control, TTFAEt alone treated, ISO control and pretreated (TTFAEt+ISO) groups. The extract was administered at a dose of 50 mg/kg body weight for 40 days orally by gavage and ISO was administered at a dose of 85 mg/kg body weight for two consecutive days intraperitoneally at an interval of 24 h. ISO induced myocardial infarction (MI) was confirmed by disturbances in serum lipid profile, heart tissue lipid peroxidation and antioxidant enzyme levels. There was a significant increase in the levels of serum total cholesterol (32.60 %), triglycerides (41.30 %), very low density lipoproteins (81.81 %), low density lipoproteins (84%) and phospholipids (38.88 %) and a significant decrease in the levels of high density lipoproteins (33.33 %) in the ISO control group when compared to normal controls. Additionally, there is a significant decrease in the levels of heart tissue antioxidant enzymes such as superoxide dismutase, catalase, glutathione reductase, glutathione peroxidase and depletion of reduced glutathione, which indicates enhanced lipid peroxidation(172 %). Pretreatment with extract significantly showed a protective effect against ISO altered lipid profile, lipid peroxidation and antioxidant enzyme levels. The present study showed therapeutic effect of TTFAEt on lipid profile and oxidative stress in isoproterenol (ISO) induced myocardial necrosis in experimental rats.

Keywords:*Tribulus terrestris* L., isoproterenol, oxidative stress, lipid peroxidation, myo cardial necrosis

INTRODUCTION

Cardiovascular diseases (CVDs) remain the principle cause of death in both developed and developing countries, accounting roughly 20 % of all deaths worldwide annually. Among various CVDs, myocardial infarction (MI) or heart attack is the leading cause of morbidity and mortality and major cause of death by the year 2020 worldwide. MI occurs when the blood supply to a part of the heart is interrupted, causing death of heart tissue. It means necrosis of a region of

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myocardium, usually as a result of occlusion of a coronary artery (Upaganlawar et al., 2011). It is most probably induced by catecholamine metabolism in particular redox reactions that results in aminochromes and adrenochromes (Dhalla et al., 2000). These oxidative reactions produce free radical (Rupp et al., 1994).

Isoproterenol (ISO) (L-β-(3,4-dihydroxyphenyl)-α-isopropylaminoethanol hydrochloride), a synthetic catecholamine, is a β-adrenergic agonist that causes severe stress in myocardium resulting in the infarct like necrosis of heart muscle (Osadchii et al., 2007). ISO develops many metabolic and morphological aberrations in the heart tissue of the experimental animals similar to those observed in human MI (Nirmala and Puvanakrishnan, 1996). Due to oxidative metabolism ISO causes excessive production of free radicals which induce lipid peroxidation (LPO) that causes irreversible damage to the myocardial membrane (Sathish et al., 2003; Mohanthy et al., 2004). ISO also causes an increase in the levels of circulatory and myocardial lipids indicating its hyperlipidemic effect.

Tribulus terrestris L., deciduous herb of the Zygophyllaceae family, is an important herb from Indian and Chinese traditional medicine literature for the treatment of various diseases especially ischemic heart diseases (Warrier et al., 1996). It also has hypoglycemic,hypolipidemic, nephroprotective, aphrodisiac and immunomodulator activities. The major therapeutic values of the extract are antihypertensive, cardioprotective and anti-inflammatory activity. In addition, the plant is used with 0.4 % composition in preparation of bramha rasayana as one of the component. We have taken up the present study to evaluate the cardioprotective effect of TTFAEt on lipid profile and oxidative stress in isoproterenol induced MI.

MATERIALS AND METHODS

Chemicals

The chemicals used in the current study were procured from Sigma Chemical Company (St. Louis, MO, USA), and SISCO Research Laboratory Pvt. Ltd, Maharastra, India.

Plant procurement

The fresh fruits of plant material were collected in and around Sri Krishnadevaraya University, Anantapur, Andhra Pradesh, India and identified by a Taxonomist and preserved in the Department of Botany.

Preparation of plant extract

After the collection of fresh fruits of *Tribulus terrestris,* they were air dried and dried fruits are fine powdered. 100 gms of powder was infused in distilled water until 1 complete exhaustion. The extract was then filtered using Whatman No. 1 filter paper and the filtrate was evaporated in vacuo and dried using rotary evaporator at 60 °C (Kandil et al., 1994). The dried extract was stored in sterile bottles until further use.

Animal ethical clearance

Local Institutional Animal Ethical Committee of our University, obtained ethical clearance for conducting experiments on animals from Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA) (Reg. no. 470/01/a/ CPCSEA, dt. 24th Aug 2001). The present work was carried out with a prior permission from Local Institutional Animal Ethical Committee.

Induction of cardiac stress in rat

Cardiac stress was induced in rats by i.p. injection of ISO at a dose of 85 mg/kg body weight for two consecutive days at an interval of 24 h. ISO was first weighed individually in eppendorf tubes for each animal according to the weight and then solubilized, just prior to injection in 0.1 ml of distilled water.

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Experimental Design

In the present study the rats were divided into four groups of six rats each:

- Normal control;
- TTFAEt alone treated (50 mg/kg body weight for 40 days);
- ISO control: (85 mg/kg body weight, once a day for two days);
- Pretreated: (TTFAEt+ISO) (50 mg/kg body weight for 40 days and then i.p. injected with ISO (85 mg/kg body weight, once a day for two days)).

The extract dose was fixed according to previous literature. Intubation was accomplished by means of a slightly bend steel intubation needle with ball like thickening terminal, attached to a syringe and extract was infused down the food pipe.

Blood collection and serum separation

Blood was collected in Eppendorf tubes from 12 h fasted rats by means of capillary tube through retinorbital flexus after second dose of ISO administration. The blood collected without added anticoagulant was allowed to clot for 30 min and serum was separated by centrifugation and used for the analysis of lipid profile.

Animal sacrifice and organ collection

After the experimental period, animals were sacrificed by cervical dislocation and immediately hearts were removed and washed thoroughly with ice-cold 0.89 % NaCl (saline). Heart of every animal was suspended in 0.15 M KCl and 10 % homogenate of heart was prepared using Potter-Elvehjem homogenizer at 0 ^{o}C in 0.1 M Tris HCl. The whole homogenate was used for estimation of reduced glutathione (GSH) and LPO. The homogenate was centrifuged in cooling centrifuge at 12000 rpm for 45 min at 0-4 ^{o}C to remove the debris and supernatant was used for antioxidant enzyme assays.

Estimation of serum lipid profile

Serum cholesterol and triglycerides were estimated using Accurex enzymatic diagnostic kit and HDL-cholesterol was estimated by using Autozyme cholesterol diagnostic kit.

Total cholesterol(TC)

Cholesterol esterase and cholesterol oxidase converts cholesterol esters into free cholesterol, fatty acids, cholest-4-en-3-one and hydrogen peroxide. In addition, hydrogen peroxide oxidatively couples with 4-aminoantipyrine and phenol to produce red quinoneimine dye which has absorbance maximum at 510 nm.The intensity of the red colour is proportional to the amount of total cholesterol (Allain et al., 1974). The assay system contains Accurex Infinite cholesterol liquid 50 mM buffer pH 6.8, cholesterol oxidase 100 IU/L, cholesterol esterase 150 IU/L, peroxidase 500 IU/L, 4-aminoantipyrine 0.5 mM, phenol 0.5 mM and cholesterol standard (200 mg%). To 0.01 ml of serum, 1.0 ml of the reagent was added, mixed and incubated at 37 ˚C for 10 min. Cholesterol standard and blank were also treated in a similar manner. After incubation, absorbance was read at 510 nm and values are expressed as mg/dL.

Triglycerides (TG)

Glycerol released from hydrolysis of TG by lipoprotein lipase is converted by glycerol kinase into glycerol-3-phosphate, which is oxidized by glycerol phosphate oxidase to dihydroxyacetone phosphate and hydrogen peroxide. In presence of peroxidase, hydrogen peroxide oxidizes phenolic chromogen to a red coloured compound which is measured at 510 nm (Fossati et al., 1982). The assay mixture consists of lipase ≥ 4 KU/L, glycerol kinase ≥ 40 U/L, glycerol phosphate oxidase≥ 5 KU/L, peroxidase ≥ 820 U/L, triglyceride standard 200 mg%. To 0.01 ml of serum, 1.0 ml of the reagent was added, mixed and incubated at 37 °C for 10 min.Triglyceride standard and water blank were also treated in a similar manner. After incubation, absorbance of the standard and serum was read at 510 nm against blank and values are expressed as mg/dL.

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Phospholipids (PL)

The assay mixture consists of trichloroacetic acid (TCA) 5 %,perchloric acid 70 %, conc. H_2SO_4 3N, sodium acetate 50 %, ammonium molybdate 2.5 %, aminonapthosulphonic acid (ANSA) 0.5 gm, sodium bisulphate 15 %, sodium sulphate 20 %**.** PL precipitate was obtained in 0.2 ml of serum by the addition of 5 ml 5 % (w/v) TCA, 1 ml of digestion mixture (50 ml distilled water, 25 ml of 70 % perchloric acid, 25 ml conc.H_2SO_4) and heated gently for about 30-45 min until the liquid becomes colourless. It was cooled,1 ml of distilled water was added, allowed to boil for 15 sec to convert pyrophosphate to orthophosphate.1 ml of 50 % sodium acetate was added and phosphorus was estimated by Fiske Subbarow method. To the above digest, 1 ml of molybdate-II reagent (2.5 % ammonium molybdate in 3N H_2SO_4), 0.4 ml of ANSA reagent (0.5 gms of α-ANSA in 195 ml of 15 % sodium bisulphate and 5 ml of 20 % sodium sulphate) was added and volume was made up to 10 ml with distilled water and absorbance was read in spectrophotometer at 680 nm.Potassium dihydrogen phosphate is used as a standard, concentration ranging from 4-80 µg, to which 1 ml of molybdate-I reagent (2.5 % of ammonium molybdate in 5N H_2SO_4), 0.4 ml of ANSA reagent were added and the volume was made up to 10 ml with distilled water and the absorbance was read in spectrophotometer at 680 nm.

Very low density lipoprotein and low density lipoprotein

VLDL and LDL were calculated using the Friedewald et al. (1972) formula as follows:

- VLDL =TG/5
- LDL =TC - TG/5 +HDL.

High density lipoprotein cholesterol (HDL)

Phosphotungstate/Mg^{2+} precipitate chylomicrons, LDL fractions and HDL fraction remains uneffected in supernatant. Cholesterol content of HDL fraction is assayed using Autozyme cholesterol diagnostic kit (Assmann et al., 1983). The assay mixture consists of 2.4 mM phosphotungstic acid, 40 mM magnesium chloride, Autozyme HDL cholesterol working solution. To 0.2 ml of serum, 0.2 ml of precipitant reagent was added, mixed and centrifuged at 4,000 rpm for 10 min to obtain a clear supernatant.To 0.05 ml of supernatant, 1.0 ml of autozyme of HDL cholesterol working solution was added, incubated for 10 min at 37 ˚C and colour developed was read at 510 nm against a blank and a standard (50 mg%) was run simultaneously. Values are expressed as mg/dL.

Lipid peroxidation

LPO in the heart tissue was determined by assaying malondialdehyde (MDA) formation according to method of Utley et al. (1967). To 1 ml of the heart homogenate, 2 ml of TCA and 4 ml of TBA were added, heated in a water bath for 30 minutes.After cooling and centrifugation, the absorbance of the supernatant was read at 535 nm. A reagent blank was prepared using water instead of tissue homogenate.The extent of LPO was expressed as nmole MDA formed/mg protein, using a molar extinction co-efficient of MDA as $1.56x10^5$ M^{-1} cm^{-1}.

Superoxide dismutase (SOD)

SOD activity was measured based on the ability of the enzyme to inhibit the autooxidation of pyrogallol.A modified procedure described by Marklund and Marklund (1974) was adopted as followed by Soon and Tan (2002). The assay system contained 2.1 ml of buffer, 0.02 ml of enzyme source (35 µg protein) and 0.86 ml of distilled water. The reaction was initiated with 0.02 ml of pyrogallol and change in absorbance was monitored at 420 nm. The percent inhibition was calculated on the basis of comparison with a blank assay system.One unit of SOD was defined as that amount of enzyme required to inhibit the autooxidation of pyrogallol by 50 % in standard

assay system of 3 ml.The specific activity was expressed as units/min/mg protein read in spectrophotometer.

Catalase (CAT)

Catalase catalyses the break down of H_2O_2 to H_2O and O_2 and the rate of decomposition of H_2O_2 was measured spectrophotometrically at 240 nm following the method of Beers and Sizer (1952). The assay system contained 1.9 ml sodium phosphate buffer (0.05 M, pH 7.0), and 1.0 ml H_2O_2. The reaction was initiated by addition of 0.1 ml enzyme source (45 µg protein).The decrease in absorbance was monitored at 1 min interval for 5 min at 240 nm and activity was calculated using a molar absorbance index of H_2O_2. The activity was expressed as nmoles of H_2O_2 decomposed/min/mg protein.

Reduced glutathione (GSH)

Total GSH content was measured by following Ellman's method (1959). This method was based on the development of a yellow colour, when 5,5`-dithio-bis(2-nitro benzoic acid) (DTNB) reacts with the compounds containing sulphydryl groups with a maximum absorbance at 412 nm. 0.5 ml of heart tissue homogenate was deproteinized with 3.5 ml of 5 % TCA and centrifuged. To 0.5 ml of supernatant, 3.0 ml phosphate buffer and 0.5 ml of Ellman`s reagent were added and the yellow colour developed was read at 412 nm.A series of standards (4-20 µg) were treated in a similar manner along with a blank. Values are expressed as µg GSH/mg protein.

Glutathione peroxidase (GPx)

A known amount of the enzyme preparation was allowed to react with H_2O_2 in the presence of GSH for a specified time period according to the method of Rotruck et al. (1973) and remaining GSH was measured by Ellman's method (1959). To 0.5 ml phosphate buffer, 0.2 ml enzyme source, 0.2 ml GSH, 0.1 ml H_2O_2 were added and incubated at room temperature for 10 min along with a control tube containing all reagents except enzyme source. The reaction was arrested by adding 0.5 ml of 10 % TCA, centrifuged at 4000 rpm for 5 min and GSH content in 0.5 ml of supernatant was estimated. The activity was expressed as µg of GSH consumed/min/mg protein.

Glutathione reductase(GRx)

GRx catalyzes the reduction of oxidized glutathione(GSSG) by NADPH to GSH. The activity of the enzyme was measured by following the oxidation of NADPH spectrophotometrically at 340 nm according to the method of Pinto and Bartley (1969). The assay system contained 0.50 ml of potassium phosphate buffer (0.25 M, pH 7.4), 1.0 ml of EDTA, 0.10 ml of NADPH, 0.96 ml of distilled water and 0.10 ml of enzyme source (150 µg proteins). The reaction was initiated by addition of 0.24 ml of GSSG. The change in absorbance was recorded at 1 min intervals at 340 nm for 5 min. The specific activity was expressed as µmol of NADPH oxidized/min/mg protein using an extinction coefficient for NADPH of 6.22 cm^{-1} μmol^{-1}.

Glutathione-S-transferase (GST)

GST activity was measured by monitoring the increase in the absorbance at 340 nm using 1-chloro-2,4-dinitrobenzene (CDNB) as a substrate according to the method of (Habig et al., 1974).The assay system contained 1.7 ml of sodium phosphate buffer (0.14 M, pH 6.5), 0.2 ml GSH and 0.04 ml enzyme source (40 µg protein). The reaction was initiated by 0.06 ml CDNB.The change in absorbance was recorded at 1 min intervals at 340 nm for 5 min and the activity was calculated using an extinction coefficient of CDNB-GSH conjugate as 9.6 mM^{-1} and expressed as mmoles of CDNB-GSH conjugate formed/ min/mg protein.

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RESULTS

General observation and characteristics

No visible side effects and variations in animal behavior were observed when the extract is administered for 40 days, indicating the non-toxic nature of TTFAEt.

Effect of TTFAEt on serum lipid profile

Table 1 shows a significant increase ($p<0.05$) in the levels of serum TC, TG, VLDL, LDL and PL and a significant decrease in HDL-C in rats administered with ISO when compared to normal control group. Pretreatment with TTFAEt showed decreased levels of serum TC, TG, VLDL, LDL and PL and a concomitant increase in the levels of serum HDL in comparison to the ISO control group.

Effect of TTFAEt on oxidative stress and antioxidant enzymes

A significant ($p<0.05$) decrease in myocardial GSH level, along with decrease in the activities of glutathione dependent enzymes(GPX, GST, GRx), antilipid peroxidative enzymes (SOD and CAT) and increase in lipid peroxidation products in heart tissue of ISO administered rats was observed in comparison to normal control rats (Tables 2 and 3). This indicates decreased levels of tissue antioxidant status and increased lipid peroxidation in ISO induced myocardial necrosis. Pretreatment with TTFAEt significantly ($p<0.05$) prevented these adverse changes in heart tissue and maintained near normal levels.

Statistical analysis

The results were expressed as mean ± S.E.M. Data were analysed for significant difference using Duncan's Multiple Range (DMR) test ($P<0.05$) (Duncan, 1955).

Table 1: Effect of TTFAEt treatment on serum lipid profile in normal and experimental rats

Parameters	NC	TTFAEt	ISO	TTFAEt+ISO
Total cholesterol	92 ± 4.2^{a}	88 ± 4.7^{a}	124 ± 5.5^{b}	110 ± 3.7^{c}
Triglycerides	92 ± 0.3^{a}	96 ± 0.4^{a}	120 ± 0.8^{b}	105 ± 0.6^{c}
Phospholipids	75 ± 1.3^{a}	70 ± 1.3^{a}	100 ± 1.2^{b}	95 ± 1.3^{c}
Very low density lipoproteins	24 ± 0.5^{a}	20 ± 0.8^{a}	37 ± 0.8^{b}	20 ± 0.5^{a}
Low density lipoproteins	26 ± 3.4^{a}	23 ± 2.1^{a}	48 ± 3.3^{b}	38 ± 2.6^{c}
High density lipoproteins	28 ± 1.3^{a}	29 ± 2.8^{a}	20 ± 1.4^{b}	26 ± 1.3^{a}

Values are expressed as mean ± S.E.M (n=6). Means with different superscripts within the column are significantly different at P<0.05 (Duncan's multiple range test).NC: Normal control, TTFAEt: Control treated with extract, ISO: ISO control, TTFAEt+ISO: Pretreated

Table 2: Effect of TTFAEt treatment on lipid peroxidation in normal and experimental rats

Lipid peroxidation	NC	TTFAEt	ISO	TTFAEt+ISO
nmoles of MDA formed/mg of protein	23 ± 1.2^{a}	23 ± 1.8^{a}	61 ± 2.4^{b}	34 ± 2.1^{c}

Values are expressed as mean ± S.E.M (n=6). Means with different superscripts within the column are significantly different at P<0.05 (Duncan's multiple range test).NC: Normal control, TTFAEt: Control treated with extract, ISO: ISO control, TTFAEt+ISO: Pretreated

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Table 3: Effect of TTFAEt treatment on heart tissue antioxidant enzymes in normal and experimental rats

Parameters	NC	TTFAEt	ISO	TTFAEt+ISO
superoxide dismutase (U/min/mg protein)	37.4± 2.5 [a]	38.7 ± 3.1 [a]	25.4 ± 2.3[b]	31.1 ± 2.0[c]
Catalase (mmoles of H_2O_2 decomposed/min/mg protein)	76.5 ± 2.7 [a]	71.4 ± 4.3 [a]	42.6 ± 3.3 [b]	52.4 ± 2.8[c]
Glutathione (µg of GSH/mg protein)	6.4 ± 0.4 [a]	6.3± 0.5 [a]	3.7 ± 0.6 [b]	5.5 ± 0.5 [a]
glutathione peroxidase (µmoles of GSH consumed/min/mg protein)	8.5 ± 0.4 [a]	8.7 ± 0.4 [a]	3.8 ± 0.6 [b]	7.6 ± 0.2 [a]
glutathione reductase (mmoles of NADPH oxidized/min/mg protein)	31.1 ± 4.6 [a]	29.6 ± 1.7 [a]	11.7 ± 0.4 [b]	26.1 ± 0.9[c]
glutathione–S–transferase (mmoles of GSH-CDNB conjugate formed/min/mg protein)	6.0 ± 0.1 [a]	5.5 ± 0.4 [a]	3.0 ± 0.2[b]	5.0 ± 0.4[a]

Values are expressed as mean ± S.E.M (n=6). Means with different superscripts within the column are significantly different at P<0.05 (Duncan's multiple range test).NC: Normal control, TTFAEt: Control treated with extract, ISO: ISO control, TTFAEt+ISO: Pretreated

DISCUSSION

Catecholamines at low concentrations are considered to be a beneficial in regulating heart function by exerting a positive inotropic effect. Catecholamine administration at high doses or their excessive release from the endogenous stores may deplete the energy reserve of cardiomyocytes that results in biochemical and structural changes responsible for the development of irreversible damage.

Lipids play an important role in cardiovascular diseases, not only by way of hyperlipidemia and the development of atherosclerosis, but also by modifying the composition, structure and stability of the myocardium. High levels of circulating cholesterol along with TG and their accumulation in the heart tissue is usually accompanied by cardiovascular damage (Gokkusu et al., 2003). Generally, the mechanisms of actions of lipolytic hormones, including ISO, on fat cells are believed to be mediated by the cAMP cascade: lipolytic hormones activate adenylate cyclase, thereby increasing cAMP formation. The increased cAMP promotes lipolytic activity by activating cAMP dependent protein kinase, which phosphorylates hormone-sensitive lipase (Morimoto et al., 2000) and promotes higher lipid accumulation in the myocardium (Dhawan et al., 1978) by hydrolysis of stored TG. Thus ISO causes an increase in the levels of myocardial lipids indicating its hyperlipidemic effect (Radhiga et al., 2012).

In the present study, ISO evidenced its hyperlipidemic effect by increasing serum TC, TG, PL, VLDL and LDL levels and decreased levels of HDL in comparision with normal controls. High levels of LDL show positive correlation with MI, while increased levels of HDL have a negative correlation. Our earlier studies (Kareem et

al. 2009) reported hyperlipidemia in ISO induced myocardial necrosis. An increase in LDL and VLDL, along with a decrease in HDL, was observed in ISO induced rats. LDL formation occurs primarily in the catabolism of VLDL. LDL is capable of carrying the highest concentration of cholesterol and PL evidencing increased serum TC and PL (Morimoto et al., 2000). Free radicals generated by ISO promote LDL oxidation and oxidatively modified LDL is responsible for the development of atherosclerosis. HDL restricts the oxidation of LDL, thus preventing the damage to the myocardium.

Pretreatment with TTFAEt significantly decreased the increased TC, TG, PL, VLDL and LDL levels and increased the levels of HDL. This alterations in lipid profile might be due to the presence of major active constituents i.e., steroidal saponins, lignanamides and tribulosins in TTFAEt (Wang et al., 1997).

Oxidative stress, defined as a shift in the normal antioxidant balance, due to the formation of reactive oxygen species (ROS) and more probably one of the main mechanism through which catecholamine exert their toxic effects. ISO, a synthetic catecholamine induces oxidative stress similar to that of other catecholamines on oxidation by the generation of toxic reactive oxygen species (ROS) such as superoxide radicals, hydrogen peroxide radicals and hydroxyl radicals (Vaage and Valen, 1993). The model of ISO induced myocardial ischemia is considered as one of the most widely used experimental model to study the beneficial effects of many drugs and cardiac function (Wexler, 1978).

ISO induced oxidative stress rapidly generates ROS which plays a key role in the development of MI. The induced oxidative stress by spontaneous oxidation of catecholamines resulting in the formation of catecholamine-o-quinones, which generate aminochromes through cyclisation. While, adrenochrome (which results from the cyclisation of epinephrine-o-quinone) can be oxidized to several other compounds such as adrenolutin, 5,6-dihydroxy-1-methylindole (DHMI) or adrenochrome-adrenolutin dimer. All these redox reactions generate ROS (Upaganlawar et al., 2011). ROS primarily attack poly unsaturated fatty acids (PUFA), which is an initiator of lipid peroxide formation (Karthika et al.,2011). ISO induced oxidative stress enhances LPO. Increased myocardial lipid peroxides are suggestive of lipid accumulation and irreversible myocardial damage. Intense lipid peroxides caused by ISO may affect the mitochondrial and cytoplasmic membrane causing more severe oxidative damage in the heart and consequently releasing lipid peroxides into circulation (Banerjee et al., 2003). Prior treatment with TTFAEt decreased the levels of LPO products in ISO induced rats shows its antilipid peroxidation effect.

Free radical scavenging enzymes such as CAT, SOD, and GPx are the first line of cellular defense against oxidative injury, decomposing O_2 and H_2O_2 before their interaction to form the more reactive hydroxyl radical. In ISO induced rats, the activities of heart tissue antioxidant enzymes were decreased. During MI, SOD and CAT are structurally and functionally impaired by free radicals resulting in myocardial damage. The decrease in SOD and catalase may be due to the involvement of superoxide and hydrogen peroxide free radicals in myocardial cell damage mediated by ISO (Guarnieri et al., 1980). Normal activities of GSH-dependent enzymes such as GPx, GRx, and GST are vital for maintaining the antioxidant status. The decreased activity of these enzymes in the heart is due to increased LPO in ISO-induced rats. Increased LPO resulted in decreased levels of GSH. Thus, the decreased levels of GSH resulted in decreased activities of GPx, GRx, and GST in ISO induced rats. Increasing GSH content can prevent cellular damage. Thus, the observed increased content of GSH prevented cellular damage and enhanced the activities of GSH-dependent enzymes in TTFAEt pretreated ISO administered rats. The effect of TTFAEt on GSH dependent

enzymes and GSH clearly indicates the antioxidant potential of TTFAEt.

Administration of extract maintained normal endogenous antioxidant activity by increasing the levels of reduced GSH and antioxidant enzymes such as SOD, CAT and GPx along with decreased levels of lipid peroxidation product MDA by its free radical scavenging activity.

CONCLUSION

Biochemical findings of the present study indicate that TTFAEt possesses antioxidant properties in myocardium and protects against ISO induced MI. The most important protective mechanism offered by TTFAEt is through its ability to decrease lipid profile, lipid peroxidation and increased levels of antioxidant enzymes. Thus, TTFAEt has been shown to possess cardioprotective activity against ISO induced oxidative stress in rats.

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