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EFFECT OF *TRIBULUS TERRESTRIS* FRUIT AQUEOUS EXTRACT ON HYPERLIPIDAEMIA AND MAINTENANCE OF LIVER ARCHITECTURE IN ISOPROTERENOL-INDUCED MYOCARDIAL INFARCTION IN RATS

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ABSTRACT

The present study investigates the protective effects of Tribulus terrestris Fruit aqueous Extract (TTFAEt) in myocardially infarcted rats. The oral administration of TTFAEt to rats for 40 days afforded good protection against isoproterenol-induced alterations, like Hyperlipidemia: the disorders of lipid metabolism have been ranked as one of the greatest risk factors contributing to the prevalence and severity of atherosclerosis, stroke and coronary heart diseases. Cardiac levels of lipid peroxidation (LPO) as well as the activities of antioxidant enzymes like Superoxide dismutase (SOD), Catalase (CAT), glutathione peroxidase (GPx), Glutathione-S-transferase (GST). Antioxidants can prevent reactive oxygen species-mediated damage and thus may have potential application in the prevention and cure of such diseases. Myocardial infarction produces a significant abnormal liver functioning. Liver tissue marker enzymes like alanine aminotransferase (ALT), aspartate aminotransferase (AST) and lactate dehydrogenase (LDH). The protective effect of TTFAEt was further supported by the reversal of isoproterenol-induced histological changes in the liver. The results suggest that TTFAEt protect the heart and circulatory system and also hepatoprotective and thereby maintain the near normal architecture of liver tissue.

KEY WORDS: TTFAEt, cardiac lipid profile, Lipid peroxidation, Anti-oxidant enzymes, liver marker enzymes, histopathology

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INTRODUCTION

Cardiovascular diseases affect the proper functioning of the heart and blood vessels [1]. Oxidative stress is the major etiopathological factor in ISO-induced myocardial necrosis. Hyperlipidemia is characterized by elevated serum total cholesterol, low density lipoprotein, very low-density lipoprotein (LDL, VLDL) cholesterol and decreased high-density lipoprotein (HDL) levels. Therefore, the treatment of hyperlipidemia may reduce the risk of developing ischemic heart disease or the occurrence of further cardiovascular or cerebrovascular disease in patients [2]. Presently existing hypolipidemic drugs have been associated with a number of side effects [3]. The consumption of synthetic drugs leads to hyperuricemia, diarrhoea, nausea, myositis, gastric irritation, flushing, dry skin and abnormal liver function [4]. A relatively low amount of endogenous antioxidant makes the heart vulnerable to oxidative stress-induced damage [5]. Considerable efforts have been made in the exploration of the potential for exogenous antioxidants and free radical scavengers to supplement endogenous antioxidant system and limit free radical injury, with mixed success and failure [6]. One reason why exogenous antioxidants have limited success in the prevention of myocardial injury may be due to the inaccessibility of large molecules to the key intracellular sites of oxidative damage. Under such circumstances, other options need to be explored which will help in circumventing this problem whether, by any means, it is possible to stimulate or augment the endogenous antioxidant defense system of the heart [7]. One of the proposed mechanisms of action of such drugs is by enhancing basal cellular endogenous antioxidant enzymes (SOD, CAT, GSHPx), and nucleic acid biosynthesis [8].

The liver is the largest organ in the body and it is involved in various metabolic functions. Liver disease, sufficient to compromise liver function, significantly has serious secondary effects on the functions of many other organs in the body. Conversely, diseases of other organs can interfere with liver function, either directly or indirectly [9]. In cardiovascular diseases, hepatic damage always occurs [10]. Liver cells contain many enzymes that may be released into the blood in various pathological processes [11]. *Tribulus terrestris* has long been a constituent in tonics in Indian ayurveda practice, where it is known by its Sanskrit name, gokshura. It is also used as an aphrodisiac, diuretic and nervine in Ayurveda, and in Unani, another medical system of India. The saponins (furostanol) and flavanoids present in the leaves and fruits are the active principles responsible for its vasodilatory and diuretic properties [12]. First report of cardiac actions of Tribulus terrestris was published in 1976; there has been an inadequacy of research investigating the potential benefits of using TTFAEt as a cardioprotective agent [13]. Flavonoids are reported to have antioxidant and hepatoprotective properties [14].The focus of the present study was to evaluate the effect of TTFAEt on heart and liver against isoproterenol-induced myocardial infarction in albino rats.

MATERIALS AND METHODS

Tribulus terrestris was received as a gift from Chemiloids manufacturers and exporters of chemicals, alkaloids and herbal extracts, based in Vijayawada, Andhra Pradesh, India. Adult male albino rats, weighing 120-150g, were procured from the National Centre for Animal Science, National Institute of Nutrition, Hyderabad, India. The study was approved by Animal Ethics Committee of S. K University, Anantapur (REGD. No. 470/01/a/ CPCSEA, DT. 24th Aug 2001.). The rats were fed with commercial pellet rat chow (M/s. Sai Durga Feeds and Foods, Bangalore., India) and water *ad libitum* and maintained under standard laboratory conditions with 12:12 hrs light: dark cycle. The rats were divided into four groups of

six animals each. Group I rats served as positive control received 1ml of physiological saline subcutaneously (sc) for two days at end of the study, while Group II rats were pretreated with TTFAEt orally (85 mg/kg bw/day) for a period of 40 days Group III rats were administered ISO sc (85 mg/kg body weight/day) dissolved in physiological saline twice at an interval of 24 hrs for two consecutive days. While Group IV rats were pretreated with TTFAEt orally (85mg /kg bw for a period of 40 days) and then received ISO 85 mg/kg bw/ day dissolved in physiological saline sc twice at an interval of 24 hrs for two consecutive days. At the end of 40 days, the animals were fasted for 12 hours to minimize dietary effects and anesthetized with thiopentone sodium (35 mg/kg BW, ip). Blood was drawn from the external jugular vein of the rats; plasma and serum were prepared, used for the estimation of proteins [15], lipid peroxidation. Liver and the hearts were excised immediately for analysis.

BIOCHEMICAL INVESTIGATIONS

Biochemical assay in the Heart tissue

Immediately after the sacrifice, the hearts were excised and washed in ice-cold isotonic saline and blotted with a filter paper. Subsequently, the hearts were weighed. Some portion of the heart was used for homogenate the lipids were extracted by the method of Folch et al[16]. To a known volume of tissue homogenate, 10 ml of chloroform–methanol (2:1 v/v) mixture was added and mixed well for 30 min and was filtered through Whatman filter paper (No. 42) into a separating funnel. The filtrate was mixed with 0.2 ml of physiological saline and the mixture was kept overnight undisturbed. The lower phase containing the lipid was drained off into pre weighed beakers. The upper phase was re-extracted with more of chloroform–methanol mixture; the extracts were pooled and evaporated under vacuum at room temperature. The lipid extract was re-dissolved in 3 ml of chloroform–methanol (2:1) mixture and aliquots were taken for the estimation of heart tissue lipids. Total cholesterol [17], triglycerides [18], free fatty acids [19], and phospholipids [20] were assayed. A portion of the heart tissue was homogenized in 0.1M Tris–HCl buffer (pH 7.4). The homogenate was centrifuged at 7000rpm for 15 min and the resulting supernatant was used for the estimation of the following parameters such as lipid peroxide (LPO) [21], superoxide dismutase (SOD) [22], catalase (CAT) [23], glutathione peroxidase (GPx) [24], glutathione reductase (GRx) [25] and glutathione S-transferase (GST) [26] were estimated.

Biochemical assay in the liver tissue

The liver samples were homogenized in 0.1 M Tris HCl buffer solution (pH 7.5, 4°C) to give a homogenate, the tissue marker enzymes aspartate aminotransferase (AST) and alanine aminotransferase (ALT) [27] and lactate dehydrogenase (LDH) [28] were assayed.

HISTOLOGICAL EXAMINATION

Small pieces of liver tissue were flushed with normal saline, fixed in 10% formalin solution, and processed. Sections 5 m thick were cut, stained with hematoxylin and eosin, and observed under the light microscope for cell necrosis, vacuolar degenerative changes, inflammation, and fibrosis.

STATISTICAL ANALYSIS

Statistical analysis was performed by Duncan's multiple range test (DMRT). Results were expressed as mean ±S.D. For six rats in each group. P values < 0.05 were considered significant.

RESULTS

The cardiac tissue lipid profile of control and different experimental groups is presented in Table 1. In ISO administered group, the cardiac tissue TC, TG, FFA and TC to PL ratio (C/P) were significantly increased and a significant decrease in PL compared to control. Rats pretreated with TTFAEt showed significant decrease in TC, TG, FFA, and TC to PL ratio with concomitant increase in PL, compared to ISO administered rats.

Table 1
Effect of TTFAEt on cardiac tissue lipid profile in ISO-induced rats.

Parameters	Control	Treated	ISO	Pretreated
Total cholesterol	4.95 ± 0.21	5.13 ± 0.03	9.33 ± 0.11	6.11 ± 0.09
Triglycerides	4.33 ± 0.07	4.37 ± 0.14	7.49 ± 0.13	5.43 ± 0.12
Phospholipids	29.1 ± 0.25	27.7 ± 0.19	19.2 ± 0.58	29.4 ± 0.16
Free fatty acids	0.35 ± 0.06	0.37 ± 0.02	0.57 ± 0.02	0.39 ± 0.04
C/P ratio	0.170 ± 0.01	0.185 ± 0.04	0.485 ± 0.01	0.20 ± 0.02

Values are means ± S.D of six rats in each group. All the values are expressed as mg/g wet tissue, (P < 0.05, DMRT).

In Table 2, Rats induced with ISO showed a considerable increase in the levels of TBARS and LOOH in the plasma and heart compared to normal control rats. Oral pretreatment with TTFAEt to ISO-induced rats showed considerable decrease in the levels of TBARS and LOOH in plasma and the heart compared with ISO alone induced rats.

Table 2
Effect of TTFAEt on the levels of lipid peroxidation products (TBARS and LOOH) in normal and ISO-induced myocardial infarcted rats.

Parameters	Control	Treated	ISO	Pretreated
Heart TBARS (mmol/100g wet tissue)	1.51 ± 0.13	1.47 ± 0.12	8.23 ± 0.72	3.31 ± 0.29
Heart LOOH (mmol/100 g wet tissue)	28.19 ± 2.61	28.11 ± 2.63	64.53 ± 6.37	37.97 ± 3.52
Plasma TBARS (nmol/ml)	7.59 ± 0.72	7.31 ± 0.70	23.93 ± 2.05	12.95 ± 1.06
Plasma LOOH (values×10^{-5} mmol/dl)	20.59 ± 1.82	20.25 ± 1.81	45.41 ± 4.18	25.53 ± 2.34

Each value is mean ± S.D. for six rats in each group. (P < 0.05, DMRT).

The activities of enzymic antioxidants such as SOD, catalase, GPx, GRx and GST in the heart of normal and ISO-induced rats are shown in Table 3. ISO-induced rats exhibited a significant decrease in the activities of these enzymic antioxidants in the heart compared to normal control rats. Pretreatment with TTFAEt to ISO-induced rats significantly increased the activities of these enzymes compared with ISO alone induced rats.

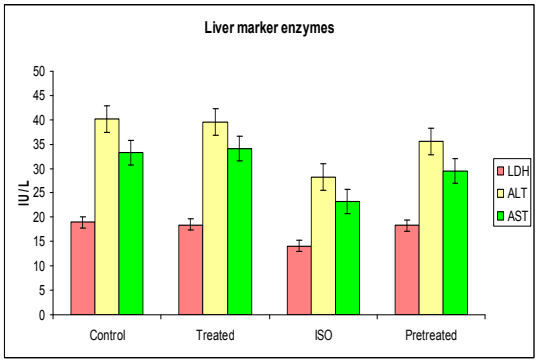
Table 3
Effect of TTFAEt on the activities of enzymic antioxidants in the heart of normal and isoproterenol (ISO)-induced myocardial infarcted rats.

Parameters	Control	Treated	ISO	Pretreated
SOD (units/mg protein)	15.63 ± 1.68	15.75 ±1.70	5.51 ± 0.56	11.47 ± 1.35
Catalase (µmol of H2O2 consumed/min/mg protein)	11.44 ± 1.21	11.70 ± 1.33	3.42 ± 0.43	10.48 ± 0.90
GPx (µg of GSH oxidized/min/mg protein)	3.65 ± 0.45	3.82 ± 0.45	2.47 ± 0.13	3.11 ± 0.40
GRx (nmol of NADPH oxidized/min/100mg protein)	7.30 ± 0.74	7.42 ± 0.81	3.17 ± 0.28	5.87 ± 0.62
GST (nmol of CDNB conjugated/min/mg protein)	757.08 ± 73.75	759.25 ± 73.87	469.83 ± 40.36	673.52 ± 58.87

Each value is means ± S.D. For six rats in each group. SOD Units: one unit is defined as the enzyme concentration required to inhibit the optical density at 560nm of chromogen production by 50% in 1min. CDNB: 1-chloro-2, 4-dinitrobenzene, (P < 0.05, DMRT).

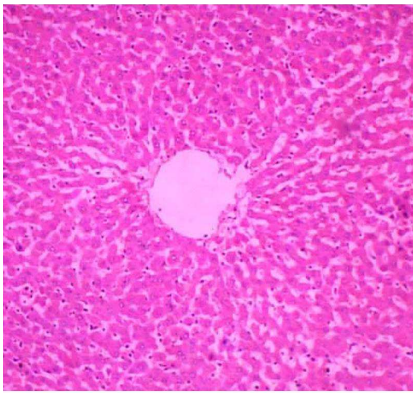
The results of the activities of Liver marker enzymes in normal and experimental group of rat liver are shown in Figure 1. Significant decrease in levels of the liver tissue marker enzymes AST, ALT and LDH were noted in isoproterenol-induced rats when compared with control rats. A slight increase in levels of the marker enzymes was noted in drug-treated rats when compared with isoproterenol-induced rats. No significant changes were noticed for drug control rats when compared with control rats. Near normal levels were maintained in drug-treated rats when compared with control rats.

Figure 1
Effect of TTFAEt on Liver Marker enzymes in ISO induced rats.

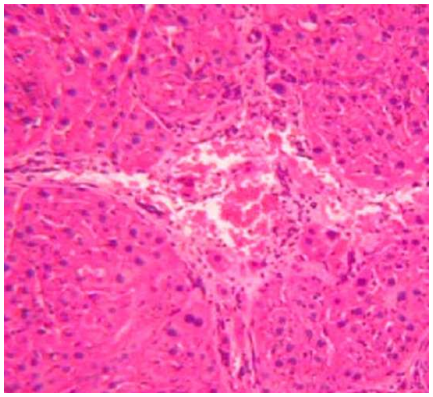


Each column is mean ±S.D for six rats in each group, (P < 0.05, DMRT).

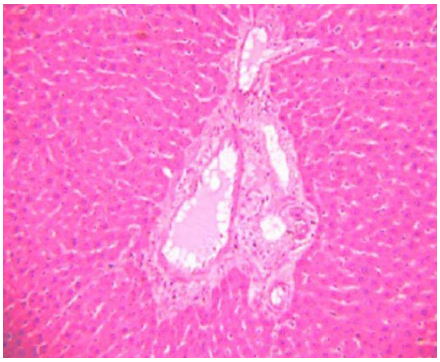
The histological observations of the liver sections in Fig. 2 are agreement with the biochemical changes. Group I showed normal architecture, group II showed near normal architecture with slight sinusoidal dilatation, group III showed inflammatory necrosis and fibrosis, and in group IV near normal hepatic architecture was restored.



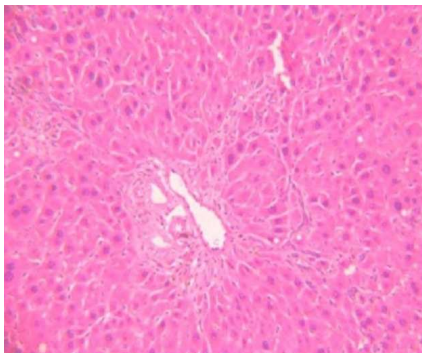
Figure, 2a
Liver tissue from a control rat shows normal architecture. Magnification X 100.



Figure, 2b
Liver tissue from a treated rat shows near normal architecture, Magnification X 100.



Figure, 2c
Liver tissue from an isoproterenol-induced rat shows areas of inflammatory necrosis and fibrosis, Magnification X 100.



Figure, 2d
Liver tissue from a pretreated rat shows near normal hepatic architecture, Magnification X 100.

DISCUSSIONS

Isoproterenol-induced myocardial oxidative stress has also been reported earlier [29]. Its metabolic effects are seen in adipose tissue, skeletal muscle, and liver. Decreased levels of tissue marker enzyme in myocardially infarcted rats can be attributed to the damaged structural integrity of the liver because these are cytoplasmic in location and are released into circulation after cellular damage. [30] Abnormalities in lipid profile are associated with increased risk of myocardial infarction. High level of circulating cholesterol and its accumulation in the heart tissue is usually accompanied by cardiovascular damage. [31]ISO-treated rats showed an increase in the levels of tissue cholesterol, triglyceride, free fatty acids and a decrease in the level of tissue phospholipids (Table 1). Our results are in agreement with previous reports.[32] Accelerated degradation of membrane phospholipids is very likely the biochemical basis for the irreversible cell injury in myocardial ischemia [33]. In this study pretreatment with TTFAEt showed a significant effect on all lipid parameters in the treatment group TTFAEt rats when compared to isoproterenol-administered rats. This protective action might be due to the effective quenching of free radicals by TTFAEt. It contains saponins, flavones, alkaloids, polysaccharides, etc. As its main ingredient, TTFAEt can lower blood lipid, prevent deposition of lipid on artery and myocardium, prohibit platelet aggregation, antagonize anoxia and protect the heart from hypoxia/re-oxygen injury [34]. Increased lipid peroxidation is thought to be a consequence of oxidative stress which occurs when the dynamic balance between prooxidant and antioxidant mechanism is impaired [35]. Reactive oxygen species (ROS) may attack any type of molecules, but their main target appears to be polyunsaturated fatty acids, which is the precursor of lipid peroxide formation.[36] [52] Elevation of lipid peroxides in ISO-induced rats could be attributed to the accumulation of lipids in the heart and the irreversible damage to the myocardial membranes. ROS are highly toxic byproducts of aerobic metabolism; react unfavorably with surrounding macromolecules resulting in severe cell and tissue damage. Pretreatment with TTFAEt to ISO-induced rats significantly decreased the levels of serum TBARS (Table 2). Flavonoids have been shown to inhibit lipid peroxidation formation in rat tissues and also inhibit the free radical production in the cells at various stages.

Antioxidants constitute the foremost defense system that limits the toxicity associated with free radicals. The equilibrium between antioxidants and free radicals is an important process for the effective removal of oxidative stress in intracellular organelles. However, in pathological conditions like MI, the generation of ROS can dramatically upset this balance with an increased demand on the antioxidant defense system. Free radical scavenging enzymes such as SOD, catalase, GPx, GRx and GST are the first line of cellular defense against oxidative injury. These enzymes are lowered due to enhanced lipid peroxidation. Superoxide radicals generated at the site of damage in MI modulates SOD and catalase resulting in the lowered activities of these enzymes and accumulation of superoxide anion, which also damages the myocardium [37]. Rats pretreated with TTFAEt showed increased activities of these enzymes which suggest that TTFAEt may have the ability to prevent the deleterious effects induced by free radicals in ISO-induced rats. GPx and GST activities are significantly depressed in ISO induced rats, which may be due to the reduced availability of GSH. Inactivation of GRx in the heart leads to accumulation of oxidized glutathione (GSSG) [38]. GSSG inactivates the enzymes containing SH-group and inhibits protein synthesis [39]. Increase in the activities of GPx, GRx and GST on pretreatment with TTFAEt in ISO-induced MI group shows the antioxidant potential of TTFAEt against injury caused by free radicals. GSH is important in protecting the myocardium against free radical mediated injury and thus reduction in cellular GSH content could impair recovery after short period of ischemia. Depressed GSH levels may be associated with an enhanced

protective mechanism to oxidative stress in MI. ISO administration was found to reduce the levels of GSH in plasma and cardiac tissue and these observations concur with earlier finding.

Measurements of aminotransferases and LDH assess hepatocyte integrity. Even mild changes in their levels may be due to the presence of potentially significant liver diseases [40 41]. Damage due to isoproterenol might have led to the leakage of these enzymes from liver tissue into the blood stream, but TTFAEt administration might have minimized the effect of isoproterenol and would have prevented the leakage, thereby maintaining the values at near normalcy in drug-treated rats when compared with control rats. ALT (in cytoplasm) and AST (in cell cytoplasm and mitochondria) occur in much higher concentration in liver than elsewhere and consequently their decrease reflects hepatic damage more specifically [42]. The hepatic histology generally correlates with the clinical or biochemical severity of cardiovascular disease. Liver dysfunction leading to the associated histological changes has long been recognized as a complication of severe heart failure as reported earlier [43]. Central hepatic necrosis associated with left-sided heart failure is recognized mostly [44]. Group I showed normal architecture. Group II showed near normal architecture with slight sinusoidal dilatation. In Group III sinusoids adjacent to the terminal hepatic veins are dilated, and hepatocytes show nuclear vacuolation. Hyaline thickening of the central vein is seen. In this form of liver damage only a small area of normal-appearing hepatocytes remains in the perivenular area. Necrosis and fibrosis are common occurrences, which is similar to earlier reports [45 46]. In the case of Group IV rat's near-normal hepatic architecture is seen.

Saponins, the active constituent of TTFAEt inhibit the metabolism of arachidonic acid via the Cyclooxygenase and lipooxygenase pathways that generates reactive oxygen species; resulting in a decrease in the levels of lipid peroxides [47]. T. terrestris Flavonoids have been shown to possess hepatoprotective activities.[48 49 51] The hepatoprotective effect of *T. terrestris* may therefore be due to the presence of flavonoids (Figs. 1). The studied plant extract contains antioxidants and hepatoprotective activity through a regulatory action on cellular permeability, stability and suppressing oxidative stress. A number of scientific reports indicated that certain flavonoids, tri terpenoids and steroids have protective effects on the liver [50].

CONCLUSIONS

The present study demonstrates that TTFAEt offered significant protection against ISO induced myocardial necrosis and hepatic damage through a unique property of antihyperlipidemic, enhancement of basal endogenous antioxidants, hepatoprotective activity through a regulatory action on cellular permeability, stability and antioxidant property without producing any cytotoxic effects.

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