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CARDIOPROTECTIVE AND ANTILIPIDEMIC ROLE OF *OCIMUM BASILICUM* SEEDS OIL AND *LINUM USITATISSIMUM* SEEDS OIL IN ACUTE MYOCARDIAL INFARCTION MALE RABBITS INDUCED BY ISOPROTERENOL

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Key word: Basil seed Oil, Flaxseed Oil. ECG, Heart rate.

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ABSTRACT

This study was undertaken to investigate the possible protective effect of *Ocimum basilicum* seeds oil and *Linum usitatissimum* seeds oil on heart function, antioxidant and lipid profile test, when induced acute myocardial infarction in rabbits by isoproterenol. Thirty-six male rabbits were divided into six groups: group (C): control negative, group (ISO): received isoproterenol (control positive), (BP) group: basil seed oil protective group, (FP) group: flaxseed oil protective group, (BT) group: basil seed oil treated group and (FT) group: flaxseed oil treated group. ECG and blood samples tropnine I, antioxidant enzymes and lipid profile were done. The analysis of ECG in rabbits treated with isoproterenol showed T wave inversion and an increase in heart rate. While rabbits treated with basil oil and flaxseed oil restored T wave and heart rate to near normal. The results of the experiment revealed that administration of basil oil and flaxseed oil in all groups caused a significant decrease ($p<0.05$) in cardiac troponin I and lipid profile (TC, TG, LDL, VLDL) and significant increase ($p<0.05$) in HDL and antioxidant enzymes Glutathione Peroxidase (GPx) and Superoxide Dismutase (SOD) compare with ISO group. The study concluded the basil oil and flaxseed oil have cardioprotective and ameliorative effects against acute myocardial infarction induced by isoproterenol in experimental animals

INTRODUCTION

Myocardial infarction commonly known as heart attack is a disease that occurs when the blood supply to a part of the heart is interrupted, causing ischemia, injury and ultimately to cell death of heart tissue. It means necrosis of a region of myocardium caused by a disruption in the supply of blood to the heart usually as a result of occlusion of a coronary artery also called as cardiac infarction (1). Myocardial infarction is a type of acute coronary syndrome it is usually characterized by varying degree of chest pain, sweating, weakness, nausea, arrhythmia and sometimes causes loss of consciousness and even sudden death (2). *Ocimum basilicum* (basil) contain a wide range of essential oils (such as lienoilic acid) is an important medicinal plant in a variety of traditional and folk systems of medicines (3). In Ayurvedic medicine basil used for stomach spasm, colds, fever, vomiting, antibiotic properties and lowers blood sugar levels. In Chinese medicine, basil is used for kidney malfunction and gum ulcers. Since basil has the ability to lower blood pressure thought to have an affinity for the heart, as well as serving the body to adapt to new demands and stresses (4,5). *Linumu sitatissimum* (flax): The main chemical composition of flax are polyunsaturated fatty acids which are an omega-3 family. Flaxseed oil has been used as a topical demulcent and emollient and as a laxative, particularly for animals and flaxseed cakes have been used as cattle feed (6). Flaxseed is also used for animal feed to improve animal reproductive performance and health (7,8). Several studies have suggested that basil and flaxseed extract improve heart function and involved in reducing lipid levels or its effects enhance of lipid-resistance to lipid oxidation(9,10). This work aimed to determine the effect of protective and ameliorative effect of *Ocimum basilicum* seeds oil and *Linum usitatissimum* seeds oil on heart function in acute myocardial infarction in male rabbits induced by isoproterenol.

MATERIALS AND METHODS

Thirty-six healthy male domestic rabbits brought from local market /Basra, weighting (1200-1450) grams. The rabbits kept under observation for 10 days. They were provided with feed and tap water *adlibitum*. Rabbits were randomly divided into six groups (6 rabbits for each group): Group C: negative control received 1ml olive oil orally for 32 days. Group ISO: Positive control received isoproterenol (70mg/kg S.C.) for 2 consecutive days. Group BP: Received 900 mg/Kg basil oil orally for 32 days. Then, on day 31 isoproterenol (70mg/kg S.C.) for 2 consecutive days. Group FP: Received 50 mg/Kg flaxseed oil orally for 32 days, then, on day 31, isoproterenol (70mg/kg S.C.) for 2 consecutive days. Group BT: Received isoproterenol (70mg/kg S.C.) for two consecutive days.

Then, received 900 mg/Kg basil oil orally for 32 days. Group FT: Received isoproterenol (70mg/kg S.C.) for two consecutive days. Then, received 50mg/Kg flaxseed oil orally for 32 days.

Preparation of Animals for Recording ECG: The rabbits were placed on a table and then immobilized by ligation the abdomen and four limbs. Then, they were left about 10 minutes to get calm. Electrodes were attached to the skin at the triceps brachii muscle of the right and left limbs and biceps femoris muscle of the right and left hips. Where the alligator clips were attached electrode, gel was rubbed into the skin, ECGs and heart rate were recorded by a direct writing electrocardiogram. All ECGs were standardized at 1mv=10mm, with a chart speed of 25mm/sec. ECG were recorded at 3^{ed}, 15^{th} and 33^{ed} days of the experiment.

Blood collection: the blood samples were collected from ear margin vein (5 ml) by using butterfly needles (23 G) at end of the experiment, Blood sample centrifuged to isolate blood serum to estimate the biochemical measurement.

Biochemical assay: The serum biochemical test determined by using commercial kits: tropoin I rapid test (Abon/China), Ichroma troponin I (Boditech/Korea), GPx (Elabscience/USA), SOD (Elabscience/USA) and Lipid profile (Spinreact/Spain)

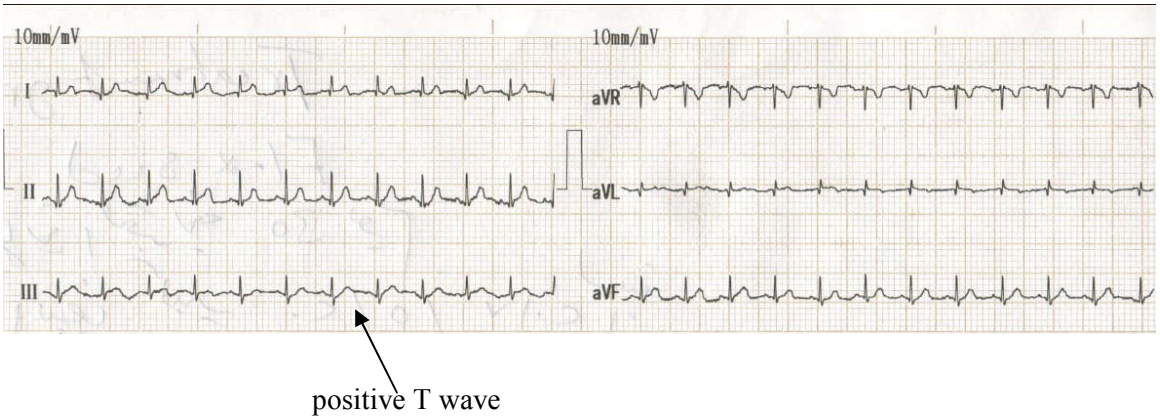
Statistical analysis of data was performed on the basis of Two-Way Analysis of Variance (ANOVA) by using computerized SPSS program version 22.0. The data were presented as mean ± stander deviation.

Result

Electrocardiograph Data (ECG)

Effect of Isoproterenol group (70mg/kg S.C) for 2 consecutive days on ECG at the pretreated period with basil oil and flaxseed oil (zero day) showed that there was marked T wave inversion (negative wave) as compared with control group (positive T wave).

C



ISO

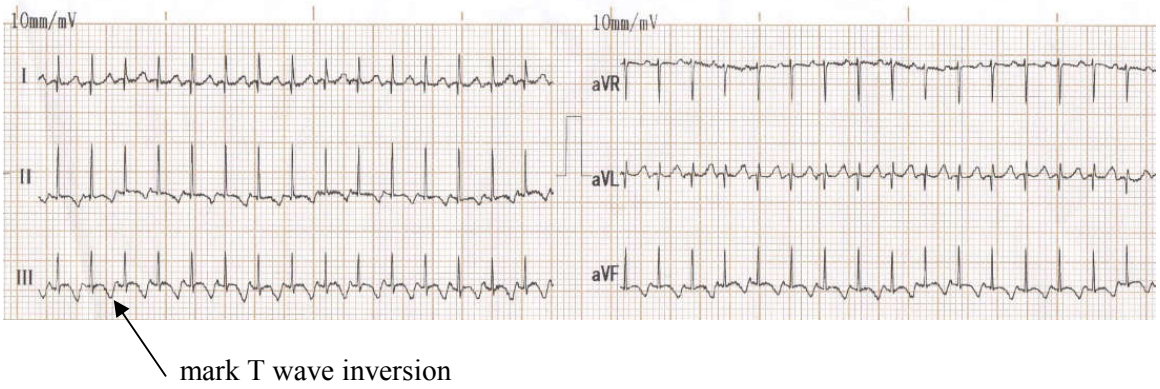
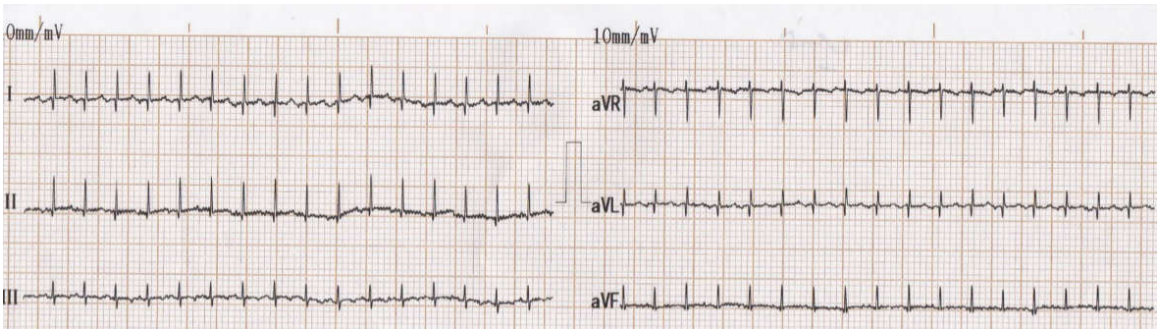


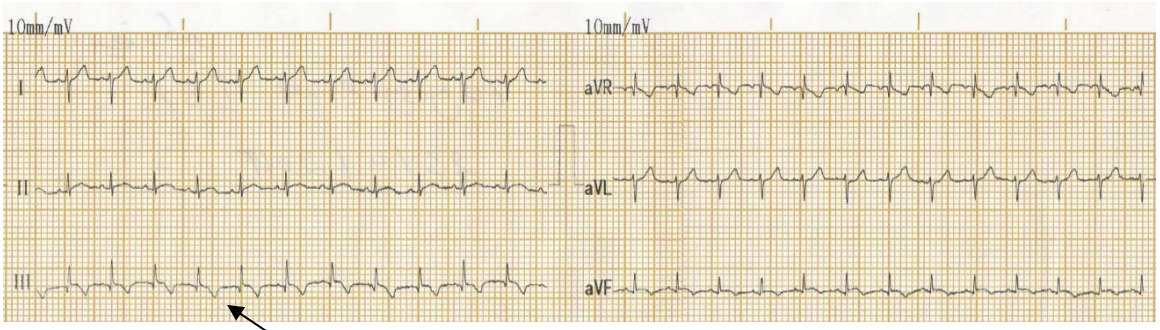
Figure (2): **Electrocardiograms of the control group (C) with normal patterns and isoproterenol group (ISO) appear T wave inversion (negative wave) in lead I, II and aVF.**

Effect of basil oil and flaxseed oil administration on ECG after 15 days showed normal patterns of ECG, whereas the effect of basil oil and flaxseed oil on isoproterenol treated groups showed mild T wave inversion as compared with Isoproterenol group.

C

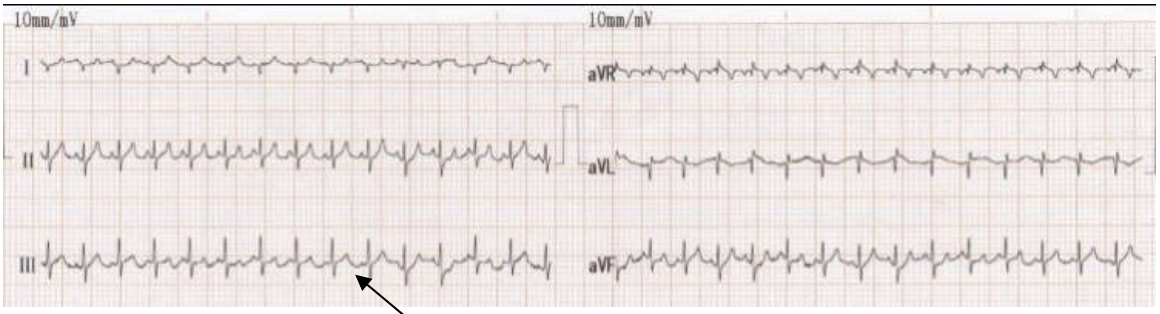


ISO



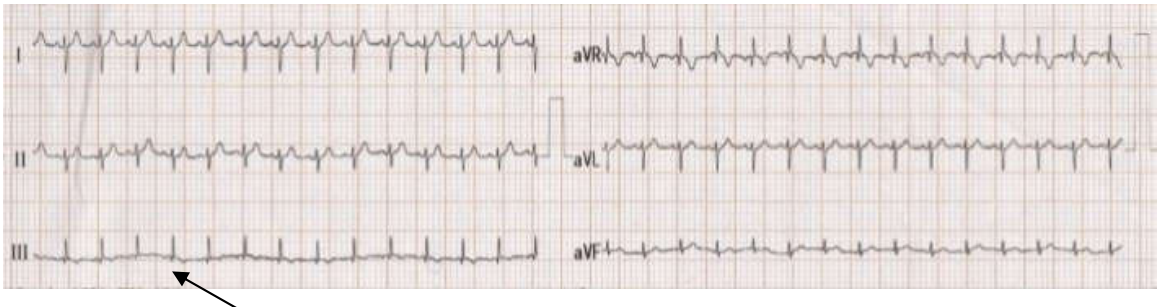
mark T wave inversion

BP



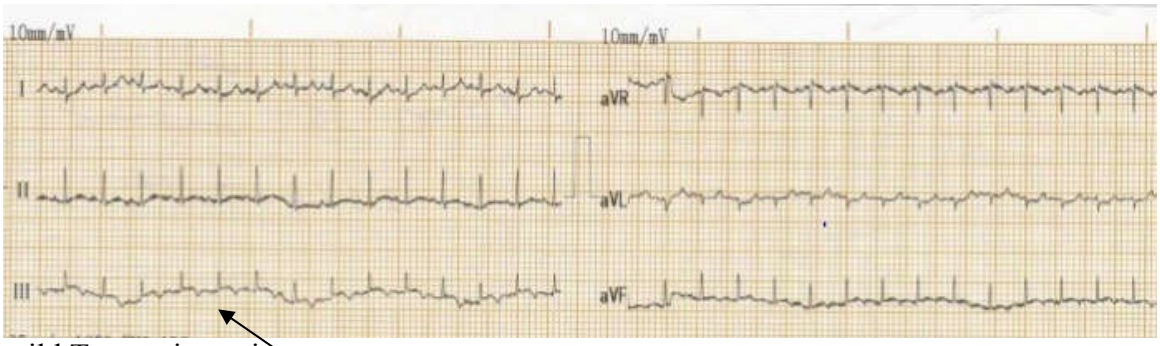
positive T wave

FP



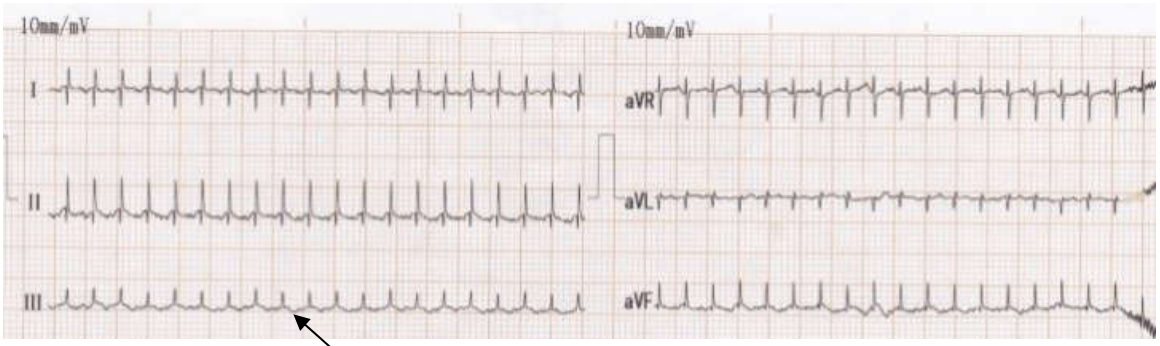
Positive T wave

BT



mild T wave inversion

FT

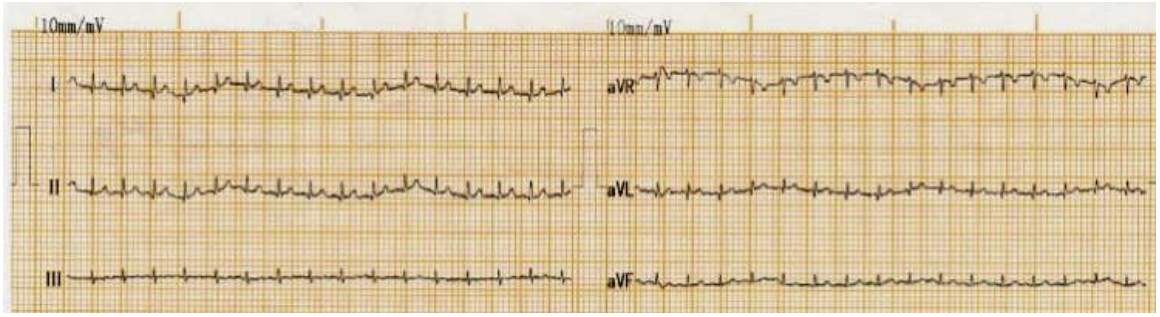


mild T wave inversion

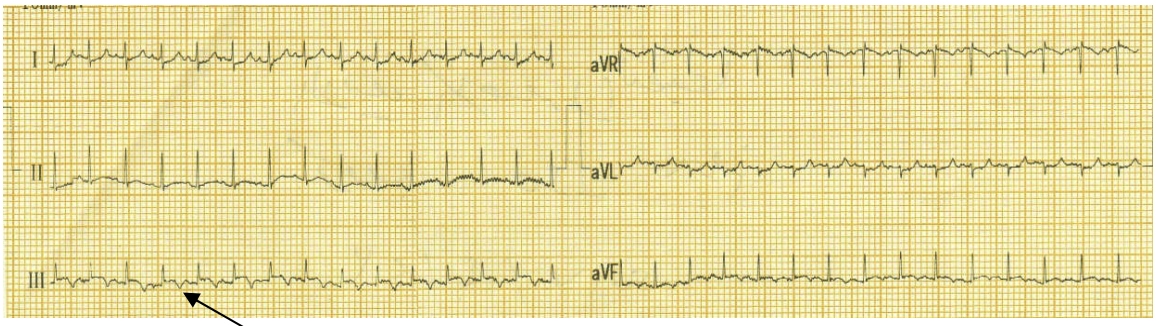
Figure (3): Effect of basil seed oil and flaxseed oil on ECG patterns in isoproterenol treated rabbits in day 15: (C) control, (BP) basil seed oil and (FP) flaxseed oil showing normal ECG pattern. (ISO) isoproterenol treated show marked T wave inversion lead II. (BT) basil oil+ Isoprenaline and (FT) Flaxseed oil +Isoproterenol showing mild T wave inversion.

Effect of basil oil and flaxseed oil administration on ECG after 33 days showed slight changes (T wave inversion) on a pattern of ECG in group BP and group FP after injection of two consecutive doses of isoproterenol when compared with ISO group. While BT and FT group showed restored T wave to near normal as compared with control group.

C

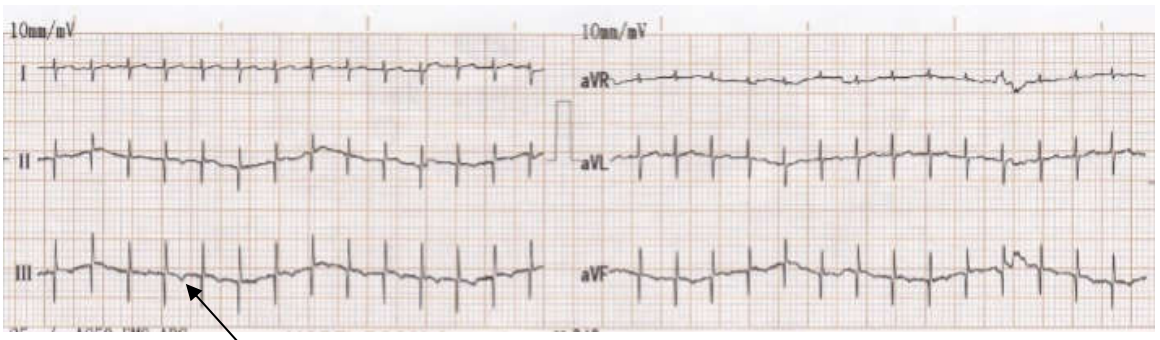


ISO



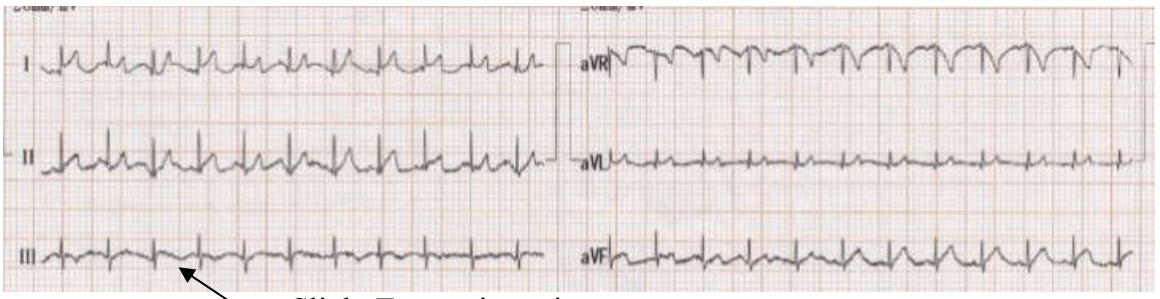
T wave inversion

BP



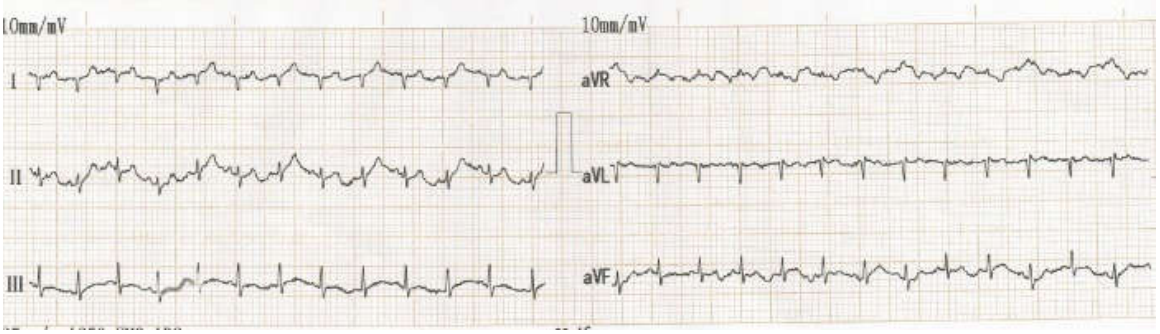
slight T wave inversion

FP



Slight T wave invertion

BT



FT

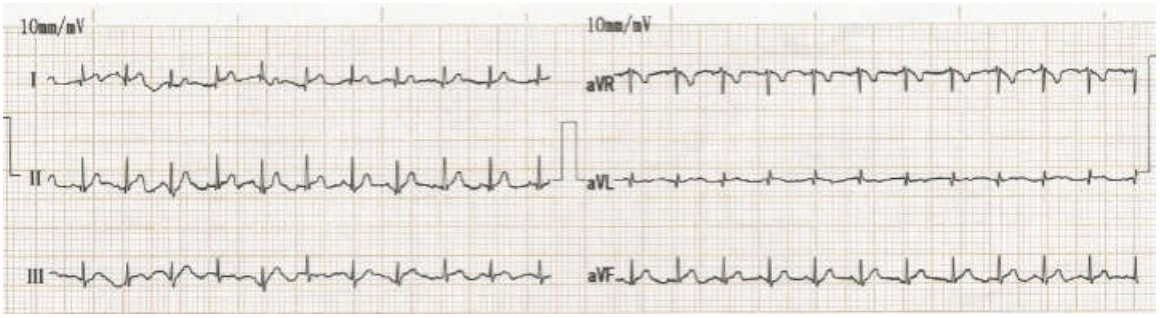


Figure (4): Effect of basil seed oil and flaxseed oil on ECG patterns in isoproterenol treated rabbits in day 32:(C) control, (BP) basil seed oil and (FP) flaxseed oil showing mild changes in T wave pattern. (ISO) isoproterenol treated show marked T wave inversion lead II. (BT) basil oil+ Isoproterenol and (FT) Flaxseed oil +Isoproterenol showing T wave near normal pattern.

The effect of oral administration of rabbits with (900 mg/Kg) basil oil and (50 mg/Kg) flaxseed oil on heart rate explained in a (Table -1). There was a significant decrease ($P<0.05$) in heart rate data in BP and FP group as compared with ISO group on day 33after injection with isoproterenol (70 mg/Kg) for 2 consecutive days whereas no statistics differences with control. While administration of basil oil and flaxseed oil to AMI rabbits (BT and FT group) showed a significant decrease ($P<0.05$) in heart rate data compared to ISO group in 33 days and no significant difference with control.

Table (1): Effect of basil oil and flaxseed oil extract on heart rate in isoproterenol-induced acute myocardial infarction in male rabbits

Parameter	Treatment					
	C	ISO	BP	FP	BT	FT
Heart Rate b/m	222 ±2.85 B	279 ±3.07 A	247 ±10.9 B	242 ±12.1 B	227 ±4.63 B	226 ±4.63 B

Values express as mean ± SD., n = 6/group. Capital letters denote difference between groups $P<0.05$. C: control group, ISO: isoproterenol, BP: basil oil, FP: flaxseed oil for 30 days then received isoproterenol, BT: AMI animals received basil oil, FT: AMI (Acute myocardial Infarction) animals received flaxseed oil.

The results of serum troponin I concentration are presented in (Table -2). The effect of daily administration of basil oil and flaxseed oil showed a significant decrease ($P<0.05$) in cT-I concentration in the BP, FP, BT and FT groups when compared with ISO group and no significant differences with the control group at the end of experiment.

Table (2): Effect of Basil Oil and Flaxseed Oil Extract on Serum cTn-I Concentration on Isoproterenol Induced Acute Myocardial Infarction in Male Rabbits.

Parameter	Treatment					
	C	ISO	BP	FP	BT	FT
Troponin I ng/ml	0.56 ±0.02 B	4.47 ±1.26 A	1.39 ±0.99 B	1.47 ±1.15 B	1.13 ±0.74 B	1.28 ±1.01 B

Values express as mean ± SD., n = 6/group. Capital letters denote difference between groups P<0.05. C: control group, ISO: isoproterenol, BP: basil oil, FP: flaxseed oil for 30 days then received isoproterenol, BT: AMI animals received basil oil, FT: AMI (Acute myocardial Infarction) animals received flaxseed oil.

The effect of basil oil and flaxseed oil on antioxidant parameters in AMI rabbits has been presented in the (Table-3). The results showed a significant increase (P<0.05) on GPx in BP, FP, BT, and FT groups compared with ISO group at the end of experiment and reach the normal levels compared with control.

The serum SOD concentration results revealed significant decrease (P<0.05) in BP and FP group treated with basil oil and flaxseed oil compared with control and there was a significant increase (P<0.05) in these groups when compared with ISO group on day 33. However, the concentration of SOD a significant increase (P<0.05) in BT group at the end of experiment days and a significant increase (P<0.05) in FT group at 33 days of the experiment compared with ISO group.

Table (4): Effect of Basil Oil and Flaxseed Oil Extract on Serum GPx and SOD Concentrations on Isoproterenol-Induced Acute Myocardial Infarction in Male Rabbits

Parameter	Treatment					
	C	ISO	BP	FP	BT	FT
GPx U/L	4895.3 ±469 A	2232.3 ±335 B	4840.7 ±455 A	4520.3 ±383 A	4588.0 ±325 A	4705.9 ±280 A
SOD U/L	63.8 ±8.3 A	34.8 ±5.3 C	51.6 ±8.7 B	53.1 ±4.5 B	46.8 ±7.8 B	41.9 ±7.2 B

Values express as mean ± SD., n = 6/group. Capital letters denote difference between groups P<0.05. C: control group, ISO: isoproterenol, BP: basil oil, FP: flaxseed oil for 32 days then received isoproterenol, BT: AMI animals received basil oil, FT: AMI animals received flaxseed oil.

The table (5) showed a significant decrease (P<0.05) in serum TC, TG, LDL and VLDL concentration was observed in group BP, FP, BT and FT at the end of the experiment compared to ISO group and there were no significant differences of TC and LDL value compared with control. While there was a significant increase (P<0.05) in TG and VLDL in BP and FP compared with control group. HDL concentration data revealed that a significant increase (P<0.05) in BP, FP, BT, and FT group and within a group when compared with ISO group at the end of the experiment and a significant decrease (P<0.05) as compared with control except for BT group.

Table (5): Effect of basil oil and flaxseed oil extract on serum TC, TG and HDL concentration on isoproterenol-induced acute myocardial infarction in male rabbits.

Parameter	Treatment					
	C	ISO	BP	FP	BT	FT
TC mg/dl	97.41 ±8.56 B	163.5 ±21.87 A	107.17 ±26.21 B	96.94 ±8.34 B	95.88 ±22.64 B	111.4 ±25.7 B
TG mg/dl	55.31 ±16.14 C	240.27 ±14.23 A	109.33 ±12.59 B	105.40 ±14.03 B	68.18 ±24.18 C	63.9 ±38.8 C
HDL mg/dl	40.89 ±0.66 A	23.68 ±2.08 C	32.42 ±4.55 B	29.14 ±3.09 B	39.53 ±4.11 A	29.43 ±1.74 B
LDL mg/dl	46.79 ±9.28 B	88.8 ±24.10 A	44.83 ±17.11 B	46.39 ±9.80 B	65.16 ±27.9 B	69.75 ±21.0 B
VLDL mg/dl	11.06 ±3.22 C	49.25 ±1.47 A	21.86 ±2.52 B	21.05 ±2.80 B	12.72 ±4.09 C	12.78 ±1.47 C

Values express as mean ± SD., n = 6/group. Capital letters denote difference between groups P<0.05. C: control group, ISO: isoproterenol, BP: basil oil, FP: flaxseed oil for 32 days then received isoproterenol, BT: AMI animals received basil oil, FT: AMI animals received flaxseed oil

DISCUSSION

The isoproterenol administration caused T wave inversion, in electrocardiographic tracings as reported in Figure (2) this finding agreement with(10, 11; 12). Normally the T wave represents the period of ventricular repolarization and the ventricular muscle fibers begin to relax that occurs during ventricular diastole. Mild ischemic is the most common cause of shortening of depolarization of cardiac muscle because of this increases current flow through the K^{+2} channels. When the ischemia happens only in one area of the heart the depolarization period of this area reduces out of proportion to that in other portions. As a result, obvious changes in the T wave can take place and T wave inversion occurs because ischemic tissue dose not depolarize normally (13).

The myocardium is composed of three electrical layers: the endocardium, the epicardium, and the M-cell layer located within the mid myocardium, each of these layers has special electrical properties and a different action potential. The M cell display a significantly longer action potential duration than the pericardial and endocardial cell types and synchronize with the end of the T wave (14). hypothesized that when myocardial infarction destroys endocardium, the long action potential of the M-cell layer can dominate the ECG producing a markedly inverted T wave and very long QT interval which are thought to reflect ischemic stunning of the subendocardium (15).

The ECG tracing of rabbits administrated with basil oil and then induced with isoproterenol on day 31 and 32 days and in group flaxseed oil and then isoproterenol on day 31 and 32 days showed mild T wave inversion on 33 day compared with ISO group and the AMI animals (BT and FT group) on basil oil and flaxseed oil treated showed an obvious improvement in their ECG pattern through the period of experiment, indicating its protective effects on cell membrane function. The results agree with (16) who found that pretreatment rats with basil oil before epinephrine administration inhibited epinephrine induced ST-segment elevation because the basil oil has the potential effects to protect the membrane of the myocardial cell.

Muralidharan, *etal* (17) noted that the alcoholic extract of basil produced marked negative chronotropic and positive ionotropic actions on frog heart, and attributed it cardiotonic effect to decrease in membrane Mg^{+2}ATPase and increase in Ca^{+2} and Na^{+}/K^{+}ATPase. While The aqueous extract produced positive ionotropic and positive chronotropic effects. so the aqueous and alcoholic extracts respectively produced The cardiotonic and ß-adrenergic effects (17,10).

On the other hand, (18) suggest that dietary 3-n intake is associated with cardiac electrophysiology in humans including slower atrioventricular conduction and ventricular repolarization, with potential implications for arrhythmic risk. Moreover, (19) observed that omega-3 PUFA infusions to exercising dogs highly susceptible to ischemia-induced fatal cardiac ventricular arrhythmias was

shortening of the QT interval and prolongation of the electrocardiographic atrial-ventricular conduction time.

Out of the results, there was a significant reduction in the heart rate in the BP, FP, BT and FT group treated with basil and flaxseed oil respectively compared with ISO group. This finding agrees with previous studies that showed the consumption of omega-3 PUFA is associated with increased heart rate variability and decrease heart rate at rest, during stress and in myocardial ischemia (20,21,22).

A previous study (23) showed that improvements in heart rate after n-3 polyunsaturated fatty acids supplementation for men with a history of myocardial infarction for the 2-4 month, is due to altered autonomic balance and a modification of the kinetic properties of voltage-gated myocardial ion channels. Moreover, (24) suggest that heart rate reductions may result from changes in cardiac autonomic regulation (improved parasympathetic and/or reduced sympathetic activity) and/or from alterations in intrinsic pacemaker rate.

Also, (20) report that n-3 Polyunsaturated fatty acids ingestion or intravenous administration reduce heart rate suggestive of an increase in cardiac parasympathetic regulation. On the other hand, the reduction in heart rate appeared larger in trials with longer duration of intake, this may relate in part to the time required for EPA and DHA to be integrated into the tissues where they exert their effects and suggests that regular consumption over time may have larger effects than short term intake (25). Similar reductions in heart rate due to omega-3fatty acids have been observed in experimental animals in rat and rabbit model. These findings suggest that omega fatty acids effect on heart rate at the level of the myocardium itself and are consistent with the concept that the voltage-gated ion channels that control the pacemaker currents in the heart (26,27).

A study in 2012 concluded that omega-3 fatty acids significantly reduce membrane electrical excitability of the cardiac myocyte by decreasing its resting membrane potential and the duration of the refractory period throughout inhibition of ion channels. These actions may be the principle mechanisms for the omega-3 fatty acid-induced reduction of heart rate observed in both humans and animals (28).

Troponin I are released to the plasma and their level rises when there is a cardiac muscles damage (29). The results of the present study revealed significant decrease in cT-I in the groups treated with basil oil and flaxseed oil respectively when compared with ISO group these results are in agreements with (16) who showed that the basil oil with vitamin E in combination decrease cTn-I concentration and may have therapeutic and prophylactic value in rats affected by myocardial infarction. Also, (30) found that S/C injections of isoproterenol induced myocardial infarction in rats. However, the

supplementation of flaxseed oil to infarcted rats induced improvement in cTn-I which supports the ethnopharmacological use of linseed oil in preventing cardiovascular diseases.

The results indicated to a significant increase in serum Gpx and SOD in all groups treated with basil oil and flaxseed oil compared with ISO group (Table-4) results may be due to the presence of omega PUFA and which acts as the antioxidant agent and neutralizing free radicals. These finding similar to that reported by (31,32, 33). During acute myocardial infarction, superoxide radicals modulate the activity of superoxide dismutase resulting in reduced activity of this enzyme and accumulation of superoxide radicals with subsequent damage to the myocardium. Glutathione is involved in decrease free radical hydrogen peroxide resulting in decreased activity of GPx in the heart of myocardial infarction induced rats (34). The oxidative stress may be by quinine metabolites of isoproterenol which react with oxygen to produce superoxide anions and others reactive oxygen species and interfere with superoxide dismutase, glutathione reductase and ATP pumps (35).

The formation of reactive oxygen species plays a principle role in cardiac pathophysiology. Therefore, the treatment of myocardial infarction can be virtually improved by targeting oxidative stress (36). The present study agreement with (16) who reported that oral administration of basil oil was able to improve and restore the level of endogenous antioxidants (GPx and SOD) in the serum as compared to MI rats.

A new research in 2017 (37) reported that linalool (omega 6) restored the level of GPx and SOD in affected cardiac tissue to a major extent. This explains that linalool could exert its action to protect the myocardial damage by possibly restricting the generation of free radicals which can prevent the necrosis of the tissue and will be restored tissue activity.

The present study noticed that isoproterenol caused a significant increase in all parameters of lipid profiles (total cholesterol, triglyceride, LDL and VLDL) and a significant decrease in HDL compared with control, indicating isoproterenol induced hyperlipidemia, which consistent with (38,39). The main causative side of isoproterenol induced hyperlipidemia a highly oxidative metabolite of catecholamines like isoproterenol accelerates the rate of peroxidation in membrane phospholipids and libration of free fatty acids into plasma by the action of phospholipase A2 and increased generation of oxidized LDL which the main factor in the vascular damage associated with high cholesterol levels (40,41).

Whereas, (42) suggested that an increased mobilization of LDL-cholesterol into the myocardial membranes from the blood may have resulted in abnormal deposition of cholesterol in the

myocardium, and the plasma concentration of atherogenic LDL-cholesterol regulated by VLDL production rate and the LDL utilization by LDL receptors (43).

Treatment different groups of rabbits with basil oil and flaxseed oil decreased isoproterenol induced a high level of lipids. The same trend of lipid profile was observed in many previous findings (44, 45). Also, (46) noted that basil a decrease in cholesterol, triglyceride, LDL and VLDL and an increase in HDL level. These effects may be due to a hypolipidemic effect of *Ocimum basilicum* via the inhibition of the key enzymes in triglycerides and cholesterol synthesis or increasing cholesterol excretion through bile acid formation.

Another scientific report has demonstrated that the main mechanisms of *Ocimum basilicum* in reducing lipid levels or its effects increase of lipid-resistance to lipid oxidation occur by some co-factors such as Cu^{2+}. Moreover, basil extract has the ability to reduce foam cell formation due to a reduction of cholesterol synthesis and the modulation of the activity of surface scavenger receptors (47). As well as holy basil can dissolve the cholesterol accumulated in the arteries (42).

A new study in 2016 (48) showed that flaxseed oil might be effective in controlling cholesterolemic status and ameliorative dyslipidemia and has the potential in reducing cardiovascular complications caused by hypercholesterolemia. Authors said that dietary flaxseed may inhibit atherosclerosis due to a decrease of circulating cholesterol levels and at a cellular level by anti-proliferative and anti-inflammatory actions (49). Essential oils are a new option of bioactive substances for cardiovascular drugs in animal models, the common properties of these substances are lipid solubility and volatility (50).

In addition, (51) recorded that omega-3 fatty acids moderately influence plasma lipid concentrations and stimulate fatty acid oxidation in the liver through peroxisome proliferator activated receptor alpha. While, (52) showed that flaxseed supplementation reduced total cholesterol, LDL, apolipoprotein B (ApoB) and apolipoprotein E (ApoE) cholesterol.

Previous research in 2010 (53) attributed the effect of n-3 PUFA in a lowering of plasma triglyceride concentrations done by a combination of an increased clearance of circulating triglycerides and a decrease inhepatic synthesis of triglycerides.

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