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Research Article

Cardioprotective and Hypocholesterolemic Effects of Ethanolic Extract of *Mormodical charantial* in Isoproterenol-Induced Myocardial Infarction in adult Rats

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Abstract

The present study was carried out to evaluate cardioprotective effect of ethanolic extract of *Mormodical charantial* in isoproterenol (ISO) induced myocardial infarction(MI) in Wistar rats. The ethanolic extract of the plant of *Mormodical charantial and* standard drug, metoprolol were prepared in normal saline and then administered orally to rats at the doses of 250 and 100mg/kg body weight (b.wt) respectively for a period of thirty days .ISO was freshly prepared in normal saline and was then used to induce MI by intraperitoneal injection at the dose of 100mg/kg to Wistar rats on the 30th day. Serum lipid profile and cardiac marker enzymes such as creatine phosphokinase (CK-MB) Isoenzyme, lactate dehydrogenase (LDH), alanine transaminase (ALT)and aspartate (AST) were obtained in the serum and in the heart homogenate of the experimental rats and then measured calorimetrically. The results show that isoproterenol-induced myocardial infarction were associated with significant ($p<0.05$) increase in the activities of cardiac marker enzymes such as AST, ALT, CK-MB and LDH in the serum with concomitant decreases in the activities of these enzymes in the myocardial tissue as compared to control group. There were also significant ($p<0.05$) increases in serum level of total cholesterol (TC) ,triglyceride(TG), low density lipoprotein(LDL) and very low density lipoprotein(VLDL) in the group injected with isoproterenol (group ii) as compared with control group . Pretreatment with leaf extract of *Mormodical charantial* at a dose of 250mg\kg b.wt and also by Metoprolol at dose 100 mg/kg body weight significantly ($p<0.05$) prevented alteration of both the lipid profile and the activities of these cardiac marker enzymes both in the serum and myocardial tissue as compared to isopreterenol-induced control group. *Mormodical charantial* possesses cardioprotective and hypocholesterolemic effects.

Keywords: cardioprotective, isoproterenol, hypocholesterolemic metoprolol

INTRODUCTION

Myocardial infarction (MI) is a diseased condition that occurs when myocardiac tisssue oxygen demand exceeds oxygen supply and is one of the most fatal manifestations of cardiovascular diseases (Mohanty *et al 2004*). MI is a complex phenomenon affecting the mechanical, electrical, structural and biochemical properties of the heart.(Petrich et al 1996). Globally, myocardial infarction (MI) is one of the leading causes of death for both men and women arising from changes in the lifestyles in developing countries (Rajadurai and Prince 2007). Isoproterenol-induced MI serves as a well-standardised model because the pathophysiological changes that occurs upon administration of isoproterenol mimic that of human MI. (Harada *et al* 1993)

Although modern drugs are effective in preventing cardiovascular disorders, their use is often restricted because of their specific toxicities to tissue (Rajadurai and Prince 2007). Herbal medicine has been in use worldwide for the treatment, control and management of a variety of ailments since prehistoric times (Griggs, 1981; Kinghorn and Balandrin, 1993; and Kong *et al*., 2003). This practice has gained more grounds because of the ready availability of plants, very low cost of preparation and the crave to avoid the side effects of drugs. One of such plants used in the management of a variety of ailments since prehistoric times is *Mormodical charantia.* Though several of its medicinal values had been reported, more work still needs to be done to add to the wealth of knowledge of this plant., *Momordica charantia* is a climber belonging to family Cucurbitaceae, is commonly known as bitter gourd or bitter melon in English. Many of the medicinal values of *Mormodical charantia* had been reported by researchers all over the world incuding; Hypocholesterolemic and anti-oxidant potential , Immunomodulatory activity , Hypotensive and anti prothrombin activities , Analgesic and antinflammatory activites, and Antimalarial activity (Jayasooriya et al., 2000; Noguchi *et al*., 2001; Ahmed *et al*., 2001, Leung *et al*. 1987, Wang and Ng2001b, Biswas *et al*., 1991;Choi *et al*., 2002 and Kohler and coauthors 2002)). Other acclaimed medicinal properties of the plant include, Antipsoriasis , Anti-HIV activity and Anticancer activity .(Takemoto *et al*., 1982b, ;

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Lee-Huang *et al*., 1995a,b; Huang *et al*., 1999,and Basch *et al*., 2003;). (rearrange the references in by citing old ones before the recent ones) The present study was carriedout to evaluate the cardioprotective effect of ethanolic leaf extract of *Mormodical charantial* in isoproterenol induced myocardial infarction in wistar rats with reference to serum, tissues biomarker enzymes and lipid profile.

MATERIAL AND METHODS

Collection and identification of plant material: Fresh leaves of *Caesalpiniabonduc* were collected in June 2007, from Igueben in Igueben Local Government Area of Edo State,Nigeria. It was identified and authenticated by Ugbogu O.A. and Shasanya O.S. of the Forest Research Institute of Nigeria (FRIN), Ibadan. The plant specimen was deposited at the FRIN Herbarium.

Chemicals: Isoproterenol and adenosine triphosphate were obtained from Sigma Chemical Company, St. Louis,MO, USA, and all other chemicals used were of analytical grade.

Processing & Extraction of crude powdered sample: The fresh leaves were air dried and the dried material was ground into powder using an electric blender (pyeUnicam, Cambridge, England). The powdered plant (60g) material was extracted with 90% ethanol using Soxhlet apparatus . Dried ethanolic extracts were obtained after removing the solvent by evaporation under reduced pressure using Rotary evaporator. The extract was stored in an air-tight container and kept in the refrigerator at 4°C until further analyses.

Animals: Swiss albino mice weighing 25-30 g and adult, Wistar rats weighing 200-250 g were procured from the Animal House, Department of Pharmacology, Faculty of Pharmacy, University of Ibadan. Food and water were provided *ad libitum.* Animals were exposed to controlled environmental temperature (28± 2°C), relative humidity (50± 5%) and 12-hour light or dark. The handling procedures were conducted in accordance with the Faculty of Pharmacy, University of Benin Ethical committee on experimental animals. The animals were also allowed two weeks under these conditions to acclimatize before the commencement of the experiments.

Acute Toxicity Studies: Acute oral toxicity (AOT) of *Mormodical charantial* was determined using Swiss albino mice in a method described by Lorke, 1983. The animals were fasted for 12 hours (overnight) prior to the experiment. The animals were divided into five groups of five animals each and were administered with single dose of extracts dissolved in 5 % tragacanth orally at doses of 1, 2, 4 ,8 and 12g/kg body weight. The animals were observed for mortality up to 48 hour (acute) and for another 14 days for subchronic toxicities.

Drugs usage: Isoproterenol drug used for the study was freshly prepared in saline at dose of 100 mg/kg body weight before each experiment. The standard drug metoprolol and the plant extract were prepared at doses of 100 and 250 mg/kg body weight respectively.

Cardioprotective Activity: The rats were divided into five groups, each group consisting of six animals.

Group 1: (Control) received only saline daily for a period of 30days.

Group 2: (Isoproterenol–induced) received saline daily for a period of 30 days and freshly prepared Isoproterenol at a dose of 100mg/kg body weight intraperitoneally on the 30th day.

Group 3: (Standard) received standard drug metoprolol at a dose of 100 mg/kg body weight orally for a period of 30 days and freshly prepared Isoproterenol at a dose of 100mg/kg body weight intraperitoneally on the 30th day.

Group 4: Received ethanolic leaf extract of *M. charantial* (250 mg/kg orally) for a period of 30 days and freshly prepared Isoproterenol at a dose of 100mg/kg body weight intraperitoneally on the 30th day .

After 24 hours of the last treatment, all the animals were anaesthesized with chloroform and blood was collected via cardiac puncture. The blood was put into plain sample tubes and sera was obtained from it by allowing it to stand for 2hrs at room temperature before centrifuging at 2000rpm . The serum was used for estimation of various biochemical parameters. The heart was dissected, immediately washed in ice-cold saline and a homogenate was prepared in 0.1 MTris-HCl buffer (pH 7.4).The homogenate was centrifuged and the supernatant was used for the assay of tissue marker enzymes.

Biochemical Analysis: The serum Total Cholesterol (TC) was determined by the method of Searcy and Berquist (1960), High Density Lipoprotein Cholesterol (HDL-C) and Low Density Lipoprotein Cholesterol (LDL-C) were determined by the method of Friedwald *et al*. (1972) and Very Low Density Lipoprotein Cholesterol (VLDL-C) level was calculated using formula. Triglyceride (TG) was by the method of Tiez (1990)

Marker Enzymes Analysis: The activities of aspartate aminotransferase (AST), alanine aminotransferase (ALT) were measured spectrophotometrically by utilizing the method of Reitman and Frankel (Reitman and Franke1957), lactate dehydrogenase (LDH) by the method of King,(1965). Creatine kinase Isoenzyme (CK-MB) activities were estimated using TECO diagnostics kits by the method of Szasz, 1976.

Statistical analysis: Statistical analysis of the results was done by one way analysis of variance (ANOVA) using SPSS software followed by Dunnet's comparison test for significance. Significance was set at ($p<0.05$). Results are presented as Mean± standard diviation , MSD.

RESULTS

There were significant ($p<0.05$) increases in serum levels of TC, TG, LDL and VLDL in the group injected with isoproterenol (group ii) as compared with control (Table 1). Pretreatment with leaf extract of *Mormodical charantial* at a dose of 250mg\kg b.wt. and also by Metoprolol at a dose of 100 mg/kg body weight significantly ($P < 0.05$) decrease serum level of TG, TC, VLDL and LDL without significant changes in the level of HDL(Table 1).

Table 1:
The effect of ethanolic leaf extracts of *Mormodical charantial* on serum lipid profile in isoproterenol-induced myocardiac infarction in Wistar rats

Group	Treatment	Total Cholesterol (mg\dL)	Triglyceride (mg\dL)	High Density Lipoprotein (mg\dL)	Low Density Lipoprotein (mg\dL)	Very Low Density Lipoprotein (mg\dL)
1	Control	72.67±8.37	10.13±0.81	24.3±4.31	46.35±4.04	1.95±0.17
2	Isoproterenol (100mg/kg)	87.76±8.41[a]	30.88±4.24[a]	24.22±5.23	57.39±4.13[ab]	5.92±0.85[a]
3	Metoprolol (100 mg/kg)	64.55±3.19[b]	8.100±1.33[b]	25.13±5.95	43.49±1.52[b]	1.47±0.18[b]
4	ELEMC (250mg/kg) + Isoproterenol (250mg/kg)	65.93±7.73[b]	6.67±0.39[b]	26.65±4.77	37.97±2.95[ab]	1.27±0.08[b]

Data is expressed as Mean ±SD for the six animals in each group
[a]p<0.05 Compared with control (Group i)
[b]p<0.05 Compared with isopreterenol –induced group control (Group 2)
ELEMC = Ethanolic Leaf Extract of *M. charantial*

Table 2.
The effect of ethanolic leaf extracts of *Mormodical charantial* on the activities of serum cardiac biomarker enzymes in isoproterenol-induced myocardiac infarction in Wistar rats

Group	Treatment	Creatine Kinase Isoenzyme (IU\L)	Lactate Dehydrogenase IU\L)	Alanine Transaminase IU\L)	Aspartate Transaminase IU\L)
1	Control	236.93±6.77	92.28±5.27	17.99±3.41	27.26±5.83
2	Isoproterenol (100mg/kg)	452.01±14.58[a]	186.54±8.06[a]	31.05±3.28[a]	76.37±10.19[a]
3	Metoprolol (100 mg/kg)	76.04±9.55[ab]	127.30±5.65[ab]	25.64±1.90[a]	56.47±5.13[ab]
4	ELEMC (250mg/kg) + Isoproterenol (250mg/kg)	231.91±10.68[b]	105.51±8.82 [b]	15.59±3.14[b]	36.69±2.93[b]

Data is expressed as Mean ±SD for the six animals each group
[a]p<0.05 Compared with control (group i)
[b]p<0.05 Compared with isopreterenol –induced group control (group 2)

In this present study, isoproterenol-induced myocardiac infarction was demonstrated to be associated with significant ($p<0.05$) increase in the activities of cardiac marker enzymes such as AST, ALT, CK-MB and LDH in the serum with concomitant decrease in the activities of these enzymes in the myocardial tissue as compared to control group (Table ii and iii). Pretreatment with leaf extract of *Mormodical charantial* at a dose of 250mg\kg b.wt and also by Metoprolol at dose 100 mg/kg body weight significantly ($p<0.05$) prevented alteration of the activities of these cardiac marker enzymes both in the serum and myocardial tissue as compared to isopreterenol-induced control group (Tables 2 and 3).

DISCUSSION

Cardiovascular diseases (CVDs) such as hypertension and myocardial infarction (MI) are the leading cause of mortality in developing countries (Rajadurai and Prince 2007, Latunde-Dada, 1990). Isopreterenol –induced myocardial infarction serves as one of the most general model to study the effect of drugs and plant extracts on cardiac function because the pathophysiological changes that occurs upon administration of isoproterenol mimic that of human myocardial infarction .(Harada et al 1993) . Induction of myocardial infarction through administration of Isopreterenol has been reported to accompany free radical generation which activates adenylate cyclase action leading to enhanced cAMP production (Barman *et al*., 2013). It also causes the elevation of serum enzymes like Creatine kinase isoenzyme (Ck-MB), lactate dehydrogenase (LDH) and Aspartate transaminase(AST) and lipid profile. These parameters act as diagnostic markers to determine the severity of myocardial infarction (Dianita *et al*., 2015). These enzymes leakage from cardiomyocytes into the bloodstream are as a result of severe myocardium stress and necrosis of the heart muscles. Cardiac muscle is susceptible to this stress because of its low levels of free- radical detoxifying enzymes or molecules (Jaffe *et al*., 2006, Koti et al 2008).

Table 3
The effect of ethanolic leaf extracts of *Mormodical charantial* on the activities of tissue cardiac biomarker enzymes in isoproterenol-induced myocardiac infarction in Wistar rats

Group	Treatment	Creatine Kinase Isoenzyme (IU\L)	Lactate Dehydrogenase IU\L)	Alanine Transaminase IU\L)	Aspartate Transaminase IU\L)
1	Control	42.38±2.52	137.53±4.42	32.71±1.97	55.42±4.00
2	Isoproterenol (100mg/kg)	31.34±1.49[a]	104.83±7.73[a]	24.11±2.95[a]	30.54±1.57[a]
3	Metoprolol (100 mg/kg)	37.19±2.66[ab]	123.00±4.36[ab]	24.73±2.49[a]	44.45±2.44ab
4	ELEMC (250mg/kg) + Isoproterenol (250mg/kg)	41.18±1.48[b]	133.78±4.32 [b]	31.28±1.34[b]	47.58±2.07[ab]

Data is expressed as Mean ±SD for the six animals each group
[a]p<0.05 Compared with control (group i)
[b]p<0.05 Compared with isopreterenol –induced group control (group 2)

In this study, intraperitoneal administration of Isopreterenol at a dose of 100mg\kg b.wt. led to significant ($p<0.05$) increase in the level of serum lipids (TG, TC,LDL-C) when compared to control (Table i). The increase in lipid profile could be as a result of Isopreterenol –induced free radical generation that activate adenylate cyclase action which in turn led to enhanced cAMP production.
.This indicates Isopreterenol –induced cAMP production might be interfering with biosynthesis of cholesterol and triglyceride. Hypercholesterolemia and its accumulation in the heart tissues have been identified as a primary risk factor in the development of cardiovascular damage. This implies that reduction in the serum levels is associated with reducing risk of CVD (Onyeneke *et al*., 2008, Salter and White 1996). The value of total cholesterol does not give the ideal arteriosclerotic status of an individual as the HDL cholesterol contributes to high total cholesterol level. Rather , the ratio of serum LDL cholesterol to serum HDL cholesterol is the best indicator of the arteriosclerotic status of an individual. The lower the ratio, the lower risk (Udoh,1998). Increase in the serum TG level also leads to increase in the synthesis of cholesterol since Cholesterol is synthesized from long chain fatty acid (component of trigyceride) in the liver. (Richards *et al*., 1989; Kanter *et al*., 1985).

Pretreatment with leaf extract of *Mormodical charantial* at a dose of 250mg\kg b.wt. and also by Metoprolol at a dose of 100 mg/kg body weight significantly ($P < 0.05$) caused decreases jn serum levels of TG, TC, VLDL and LDL without significant changes in the level of HDL(Table i). The mechanism of TG and cholesterol lowering effect of the extract might be attributed to inhibitory acitivity of lipopropein lipase , triglyceride lipase and cholesterol acyl transferase .The inhibition of lipoprotein lipase and triglyceride lipase could lead to decrease in TG hydrolysis (Jahn *et al.*, 1985). Cholesterol acyl transferase is the enzyme responsible for acylation of cholesterol to cholesterol ester in liver (Matsuda, 1994). These enzymes are associated with hypertriglyceridemia (Kanter *et al*., 1985; Richards *et al*.,1989).

The serum enzymes, Ck-MB , LDH, AST and ALT serve as sensitive indices to assess severity of myocardial ischemia . Creatine kinase (CK) also known as creatine phosphokinase (CPK) is an enzyme expressed by various tissues and cell types (Wallimann *et al.,* 1994). Creatine kinase catalyzes the conversion of creatine and adenosine triphosphate (ATP) to create phosphocreatine (PCr) and adenosine diphosphate (ADP). A lactate dehydrogenase (LDH or LD) is an enzyme found in nearly all living cells , it catalyzes the reversible conversion of pyruvates to lactate (Van Eerd*et al.*, 1996). The enzyme is also expressed in various tissues like the heart , RBC (red blood cells), brain, kidneys, placenta, liver and pancreas. ALT catalyzes the convertion of alanine and glutamate to alpha ketoglutarate and pyruvate while AST Catalyzes the conversion of aspartate and glutamate to alpha ketoglutarate and oxaloacetate. ALT although initially believed to be specific for the liver has recently been shown to also be associated with endothelial dysfunction and atherosclerosis (Schindheim et al 2007). AST is also found in other tissues such as red blood cells, the cardiac tissue and the skeletal muscle. Therefore tissues specificity and catalytic activity of these enzymes make them valuable tools for evaluating tissue damage.(Sivakumer *et al*., 2007)

In this present study, isoproterenol-induced myocardiac infarction was demonstrated to be associated with significant ($p<0.05$) increase in the activities of cardiac marker enzymes such as AST, ALT, CK-MB and LDH in the serum with concomitant decrease in the activities of these enzymes in the myocardial tissue as compared to control group(Tables ii and iii). The release may reflect alterations in the membrane integrity and permeability as a response to β-adrenergic stimulation. (Nivethetha *et al.,* 2009). These findings are consistent with previous works (Nivethatha *et al.,* 2009,Gomathi *et al*., 2014). Pretreatment with leaf extract of *Mormodical charantial* at a dose of 250mg\kg b.wt and also by Metoprolol at a dose of 100 mg/kg body weight significantly ($p<0.05$) prevented alteration of the activities of these cardiac marker enzymes both in the serum and myocardial tissue as compared to isopreterenol-induced control group(Tables ii and iii) . The cardioprotective effect of leaf extract of *Mordical charantial* is probably related to membrane-stabilizing action. The decrease in the activities of cardiac enzymes in the serum of rats induced with

isopreterenol and also the increase in the tissue of induced rats may account for the cardial protective effect of *Mormodical charantial* .Further study might be required to unfold the mechanism of action of *Mordical charantial*.

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