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Comparison of *Moringa oleifera* Leaves Extract with Atenolol on Serum triglyceride, Serum Cholesterol, Blood glucose, heart weight, body weight in Adrenaline Induced Rats

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Abstract

The research work was investigated to compare the effects of *Moringa oleifera* with atenolol on serum cholesterol level, serum triglyceride level, blood glucose level, heart weight and body weight of adrenaline induced rats (AIR) in a crossover design. The pharmacologically active components responsible for above-mentioned activities were isolated from plant using bioassay guided purification approach and the structures of the compounds were proposed by the spectroscopic methods. *Moringa oleifera* leaves extract and commercial drug atenolol were administered in adrenaline induced rat through intraperitoneal (i.p) route in everyday morning for one week at a dose 30mg/155±15-gm.body weight and 0.1 mg/155±15-gm.body weight of rat respectively. Different biochemical parameters such as heart weight, blood glucose level, serum cholesterol level, serum triglyceride level, body weight and the relationship between them were measured. The dose of the marketed drug atenolol was determined according to the previous knowledge of its pharmacokinetic parameters. Clinically effective plasma concentration as a hypotensive drug was obtained after the injection of 0.1 mg /155±5 gm body weight of drug. The Moringa oleifera leaves extract made significant changes in each cardiovascular parameter after proper investigation. From the present study we revealed that the leaves extracts of *Moringa oleifera* with atenolol has got profound hypolipidemic (Figure 3) activity. Lowering of blood glucose, heart weight, and body weight (Figure 1,2 and 4) in adrenaline induced rats (. $p<0.0001$) was significant. The lowering serum triglyceride level and serum cholesterol level between leaves extract of *Moringa oleifera* and atenolol in adrenaline induced rats was very significant ($p<0.001$ & $p<0.01$).

Key words: Serum triglyceride, Serum cholesterol, *Moringa oleifera,* atenolol, adrenaline, and heart weight

Introduction

Atherosclerosis and coronary heart disease are the major health problem in developed and modern societies .A number of epidemiological investigations have shown a clear association between dietary saturated fat and atherosclerosis . The composition of human diet plays an important role in the management of lipid and lipoprotein concentrations in the blood. The importance of serum lipoprotein disturbances and abnormal lipid metabolism characterized by hyperlipidaemia or hyperlipoproteinmia as etiological factors in the development of coronary heart diseases The use of herbs as medicines has played an important role in nearly every culture on earth, including Asia, Africa, Europe and the Americas. Herbal medicine is based on the premise that plants contain natural substances that can promote health and alleviate illness. Several herbs can help to reduce high blood cholesterol concentrations (Aattar, A. A. 2006).

Moringa oleifera, locally known as shajna, belongs to the monogeneric family Moringaceae and is widely distributed in the Indo-Bangla subcontinent and cultivated throughout the tropical belt (Nikkon,et al.,2003) Different parts of this plant are used in the indigenous systems of human medicine for the treatment of a variety of human aliments. Ethanolic leaves extract of *Moringa oleifera* used as hypotensive (Nikkon,et al.,2003;Siddiqui and Khan,1968; Kirtikar and

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Basu,1984). The leaves of *Moringa oleifera* are reported to be used as a hypocholesterolemic agent, and hypoglycemic agent (Dangi, et al., 2002; Ghasi, et al., 2000; Siddiqui and khan., 1968).

Atenolol is a b_1-selective (cardio selective) beta-adrenergic receptor blocking agent without membrane stabilizing or intrinsic sympathomimetic (partial agonist) activities. Beta-adrenoreceptor blocking activity of atenolol has been demonstrated by Reduction in resting and exercise heart rates and cardiac output, reduction of systolic and diastolic blood pressure at rest and on exercise, inhibition of isoproterenol induced tachycardia, and reduction in reflex orthostatic tachycardia (Gillman, et al., 1990). The aim of our present study is to investigate the comparative effects of *Moringa oleifera* leaves extract with atenolol on serum cholesterol level, serum triglyceride level, blood glucose level heart weight, and body weight.

Materials and Method

Drugs and chemicals

Phosphate buffer, Sodium buffer, Potassium dihydrogen phosphate, Ether (Diethylether), 0.1N HCl, Acetone, Ethanol. Atenolol was a kind gift from Square Pharmaceuticals Ltd, Bangladesh.

Extraction of plant materials

The fresh leaves were collected, dried in the sun for 7days and finally in an oven below 60^0 C. The dried plant material was ground into fine coarse powder and extracted with ethanol in cold condition (Nikkon et al., 2003). In cold extraction, the coarse powder is submerged in a suitable solvent (Ethanol) or solvent mixture in a flat bottom flask at room temperature and allowed to stand for several days with occasional shaking. When the solvent become concentrated, the content is then filtered with cotton and filter paper. Evaporation of solvent in vacuum rotary evaporator affords a crude extract of the soluble components and the extract was given to the animals following the works of other investigators (Nikkon et al., 2003; Nammi et al., 2003).

Experimental design and treatment

Preparation of dose of the plant extract: Moringa oleifera leaves extract was given as 200-mg/kg-body weight of rats. The average body weight of adrenaline induced rats were measured 155±15 gm. Thus the daily single dose of *Moringa oleifera* leaves extract was 30-mg/155±15 gm body weight of rats dissolved in 0.1 ml dimethylsulfoxide (DMSO) and then diluted with saline.

Dose preparation of atenolol: The daily dose of atenolol for human is 50-mg/70kg body weight. According to the body weight the dose of atenolol required for rats was 0.1-mg/155±15 gm body weight of rats dissolved in 0.1 ml dimethylsulfoxide (DMSO) and then diluted with saline.

Experimental treatment of rats: Albino rats were purchased from International Center for Diarrheal Disease Research, Bangladesh (ICDDR, B), Dhaka, Bangladesh. Rats were allowed free access to distilled water. A cycle of light and dark (12 hours light and 12 hours dark) and a temperature of 24±2°C were maintained in the room. At first rats were anesthetized with diethyl ether and 100 µl of adrenaline was injected into rats by intraperitoneal (i.p.) injection using a 1 ml disposable syringe for consecutive five days. After inducing adrenaline, the serum cholesterol, serum triglyceride and blood glucose levels were measured and compared with that of control rats that received only normal saline (Gillman *et al*., 1990). Except serum triglyceride level the standard values of body weight, heart weight, blood glucose level serum cholesterol level were high in adrenaline induced rats when compared to control rats (Gillman, et al., 1990; Siddike and khan.,1968) Because of metabolic effects of adrenaline; serum triglyceride level was low in adrenaline induced rats (Gillman, et al., 1990). The animals used in this study were cared for in accordance with the guidelines for the animal experiment of our University.

The animals were randomly divided into four groups. Group I was consisted of control rats which received normal saline, group II was consisted of adrenaline induced e rats (AIR), group III was consisted *Moringa oleifera* leaves extract treated adrenaline induced rats and group IV was consisted of atenolol treated adrenaline induced rats to compare pharmacological activities. *Moringa oleifer* leaves extract and commercial drug nifedipine were administered through intraperitoneal (i.p) route for one week at their respective doses in every morning till the completion of investigation. Treatment was done six times for obtaining accurate result.

Description and measurement of different parameters: Before treatment different biochemical parameters such as heart weight, serum triglyceride level (STL), serum cholesterol level (SCL), blood glucose level (BGL) and body weight of group I and group II rats were measured. The rats were sacrificed to collect blood sample and heart from each rat and investigated. Collected blood samples were analyzed for the determination blood glucose level by using BioLand G-423 glucose test meter (BioLand, Germany). Then the data were compared with the standard value. Collected blood samples about 1-2 ml was centrifuged at 4000 rpm for 10 minutes to separate the serum to determine STL, SCL by measuring absorbance using UV

spectrophotometer (Shimidzu UV-1200, Tokyo, Japan), using wet reagent diagnostic kits (Boehringer Mannheim, GmbH) according to manufacturer's protocol.

Statistical analysis

In the whole animal study each group consisted of six animals. Data were expressed as mean ± SEM. Differences in mean values between experimental groups were analyzed by unpaired t test a probability value ($p < 0.05$) was considered to be significant.

Results

After completion of one-week treatment the effects of leaves extract of *Moringa oleifera* with atenolol on Heart weight, STL, SCL, BGL, and body weight were investigated in control and adrenaline induced rats (AIR).

1. Effect of *Moringa oleifera* with atenolol on heart weight in adrenaline-induced rats is represented in figure 1

The mean heart weight of control, adrenaline induced, and leaves extract of *Moringa oleifera* with atenolol treated animals (after intraperitoneal administration of a single dose) are shown in Figure.1. Hypolipidemic effect was observed in animals treated with *Moringa oleifera* leaves extracts and atenolol. To determine whether or not there was a statistically significant difference achieved by *Moringa oleifera* leaves extract and atenolol during treatment one-way ANOVA followed by DMCT was applied and compared with the AIR. A significant reduction ($P < 0.0001$) in heart weight of *Moringa oleifera* treated (Mori treated) and atenolol treated (Ate treated) animals were observed.

2. Effect of *Moringa oleifera* with atenolol on blood-glucose level in adrenaline-induced rats is presented in figure 2.

The mean blood glucose level of control, adrenaline induced, and leaves extract of *Moringa oleifera* with atenolol treated animals (after intraperitoneal administration of a single dose) are shown in Figure 2. To determine whether or not there was a statistically significant difference achieved by *Moringa oleifera* leaves extract and atenolol during treatment one-way ANOVA followed by DMCT was applied and compared with the AIR. A significant reduction in blood glucose level of Mori treated ($p<0.0001$) and ate treated ($p<0.001$) animals were observed.

3. Effect of *Moringa oleifera* with atenolol on total serum triglyceride & serum cholesterol in adrenaline-induced rats is presented in figure 3.

The mean serum cholesterol and triglyceride levels of control, adrenaline induced and leaves extract of *Moringa oleifera* with atenolol treated animals (after intraperitoneal administration of a single dose) are shown in Figure 3. To determine whether or not there was a statistically significant difference achieved by the *Moringa oleifera* leaves extract and atenolol during treatment one-way ANOVA followed by DMCT was applied and compared with the AIR. A significant reduction in serum triglyceride level of Mori treated ($p<0.01$) and ate treated ($p<0.001$) animals were observed. Similarly a significant reduction in serum cholesterol level of Mori treated *(*$p<0.01$) and ate treated ($p<0.001$) animals were observed.

4. Effect of *Moringa oleifera* with atenolol on body weight in adrenaline-induced hypertensive rats is presented in figure 4.

The mean body weight of control, adrenaline induced and leaves extract of *Moringa oleifera* with atenolol treated animals (after intraperitoneal administration of a single dose) are shown in Figure 4. To determine whether or not there was a statistically significant difference achieved by the leaves extract of *Moringa oleifera* and atenolol during treatment one-way ANOVA followed by DMCT was applied and compared with the AIHR. A significant reduction in body weight (Day1 &Day8) of Mori treated ($p<0.0001$) and ate treated ($p<0.001$) animals were observed.

Figure 1

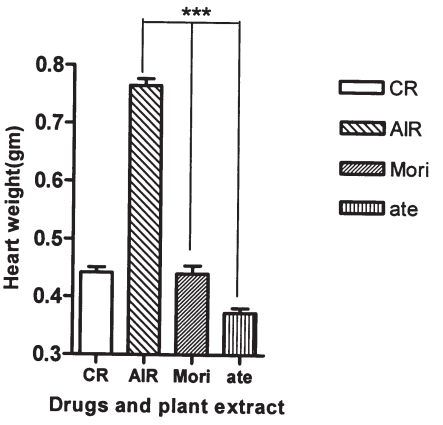

Figure1: Effect of leaves extract of Moringa oleifera with atenolol on heart weight in adrenaline-induced rats.The data are shown as mean±SEM (n=6 in each case). *** Indicates significant change in heart weight between AIR and Mori treated with Ate treated ($p<0.0001$) animals. Here AIR-Adrenaline Induced Rats; Mori-leaves extract of Moringa oleifera treated ;ate-atenolol treated and CR- control rats.

Figure2

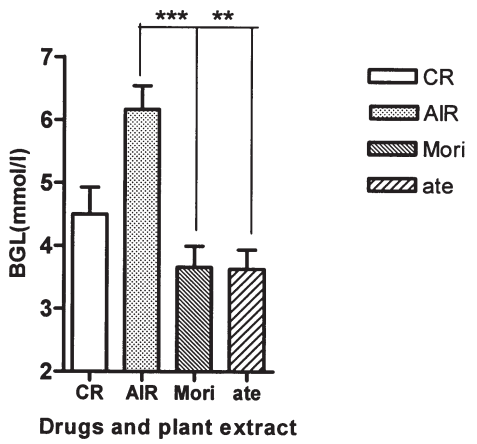

Figure 2: Effect of Moringa oleifera with atenolol on blood-glucose level in adrenaline-induced hypertensive rats. The data are shown as mean±SEM (n=6 in each case). *** Indicates significant change in heart weight between AIR and Mori treated (p<0.0001) animals, ** indicates significant change in heart weight between AIR and ate treated (p<0.0101) animals. Here AIR-Adrenaline Induced Rats; Mori-leaves extract of *Moringa oleifera* treated ;ate-atenolol treated and CR- control rats.

Figure 3

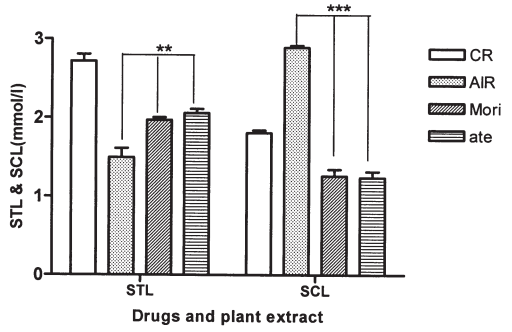

Figure 3: Effect of leaves extract of Moringa oleifera with atenolol on total serum triglyceride & serum cholesterol in adrenaline-induced rats. The data are shown as mean±SEM (n=6 in each case). **indicates significant change in serum triglyceride level between AIR and Ate treated (p<0.0010) animals, ** indicates significant change in STL between AIHR and mori treated (p<0.01) animals. Similarly *** indicates significant change in serum cholesterol between AIR and ate treated (p<0.001) animals. *** indicates significant change in SCL between AIR and Mori treated (p<0.0001) animals. Here AIR-Adrenaline Induced Rats; Mori-leaves extract of *Moringa oleifera* treated; ate-atenolol treated; CR- control rats; SCL-Serum Cholesterol level and STL-Serum Triglyceride level.

Figure 4

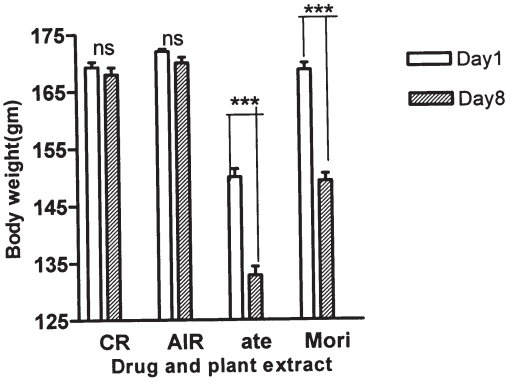

Figure 4: Effect of *Moringa oleifera* with atenolol on body weight in adrenaline-induced rats. The data are shown as mean±SEM (n=6 in each case). ***Indicates significant change in body weight (day1 and Day-8) between AIR and Mori treated (p<0.0001) and ate treated (p<0.0101) animals. The change in body weight in CR AIR was non significant. AIR-Adrenaline Induced Rats; Mori-leaves extract of *Moringa oleifera* treated; ate-atenolol treated; CR- control rats and ns-non significant.

Discussion

From the present study we revealed that the leaves extracts of *Moringa oleifera* with atenolol has got profound hypolipidemic (Figure 3) activity. Lowering of blood glucose, heart weight, and body weight (Figure 1,2 and 4) in adrenaline induced rats (p<0.0001) was significant. The lowering serum triglyceride level and serum cholesterol level between leaves extract of *Moringa oleifera* and atenolol in adrenaline induced rats was very significant (p<0.001 & p<0.01). Coronary heart disease and acute myocardial infarction is a leading cause of death due to weakening of the muscle of the heart. Cardiac dystrophy is the result of reduced blood (oxygen) transport to the heart muscle is due to the narrowing (stenosis) of the blood vessels of the arteries of the heart (Aattar, A.A. 2006). From the above result and discussion it seemed that *Moringa oleifera* leaves extract and atenolol had a greater hypolipidemic potential and may be an indication of progressive metabolic control of *Moringa oleifera* leaves extract on mechanisms involved in elimination of the lipids from the body. This study has similarity with previous investigation (Nikkon, et al, 2003; BDNF.,2001;Setoguchi,et al.,2002).However,the present data demonstrated that consumption of these leaves may be can lead to reduction in the risk of hyperlipidemic symptoms and heart diseases.

We can conclude that the plant *Moringa oleifera* may used as herbal drugs or as supplement in the treatment of

different cardiovascular complications due high lipids in the body. **T**his results should be encouraging other researcher to more work on *Moringa oleifera* including phytochemical and biological investigation especially pharmacological investigation by measuring the effect of *Moringa oleifera* on systolic and diastolic blood pressure in the treatment of hypertension although the results of present study clearly indicate those (Amran *et al*, 2004; Ara *et. al*, 2008;Boesen *et al*, 2005)), because different investigators have already been reported their popular used medicinal plant as hypotensive, anthelmic, analgesic, heart diseases, dyspepsia, ulcers (Nikkon, et al, 2003).

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مقارنة مستخلص أوراق شجرة الرواج *Moringa oleifera* مع الاتينولول على الجليسريدات الثلاثية بالمصل ، كولسترول المصل و جلوكوز الدم و وزن القلب و وزن الجسم في الجرذان المستحثة بالأدرينالين .

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الملخص

أجري هذا العمل البحثي لمقارنة تأثيرات شجرة الرواج *Moringa oleifera* مع الاتينولول Atenolol على مستوى كولسترول المصل و مستوى الجلسريدات الثلاثية بالمصل و مستوى جلوكوز الدم و وزن القلب ووزن الجسم للجرذان المستحثة بالأدرينالين (AIR) في تصميم عبوري . تم عزل المكونات الدوائية النشطة والمسؤلة عن الأنشطة آنفة الذكر من النبات باستعمال نظام التحليل الحيوي ذو التنقية الموجهة وأقترح تركيب المركبات بواسطة طرق الفحص الطيفي . تم إعطاء مستخلص أوراق *Moringa oleifera* والعقار التجاري اتينولول للجرذان المستحثة بالأدرينالين خلال التجويف البطني (i.p) صباح كل يوم لمدة أسبوع واحد بجرعة ٣٠ ملليجرام / ١٥٥±١٥ جرام من وزن الجسم و ٠,١ ملليجرام / ١٥±١٥٥ جرام من وزن الجسم للجرذان على الترتيب . تم إجراء القياسات الكيموحيوية المختلفة مثل وزن القلب و مستوى جلوكوز الدم و مستوى كولسترول المصل و مستوى الجلسريدات الثلاثية بالمصل و وزن الجسم والعلاقة بينهما . تم تحديد جرعة عقار الاتينولول المسوق وفقا للمعرفة المسبقة للمعايير الحركية الدوائية له . يتحصل على تركيز فعال سريريا في البلازما كعقار خافض لضغط الدم بعد حقن العقار بـ ٠,١ ملليجرام / ٥±١٥٥ جرام من زون الجسم . أدى مستخلص أوراق *Moringa oleifera* إلى حدوث تغيرات معنوية في كل مقياس قلبي وعائي بعد الدراسة البحثية المناسبة . اكتشفنا من الدراسة الحالية ان مستخلص أوراق شجرة الرواج مع اتينولول له نشاط واضح خافض للبيدات Hypolipidimic . انخفض معنويا ($P < ٠,٠٠٠١$) جلوكوز الدم ، وزن القلب ووزن الجسم في الجرذان المستحثة بالأدرينالين و كان الإنخفاض في مستوى الجليسريدات الثلاثية بالمصل ومستوى كولسترول المصل بين مستخلص أوراق شجرة الرواج *Moringa oleifera* والاتينولول معنويا جدا ($P < ٠,٠١$ & $P < ٠,٠٠١$) في الجرذان المستحثة بالأدرينالين .

كلمات مفتاحية : الجليسريداتت الثلاثية بالمصل ، كولسترول المصل ، شجرة الرواج *Moringa oleifera* ، اتينولول ووزن القلب .