Comparative effect of Beta blocker-Atenolol and *Murraya koenigii* (L.) *spreng* (Curry leaves) on cardiac enzyme (CK-MB) level in male albino rats

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Abstract: *Murraya koenigii* (L.) *spreng* (curry leaves) have traditionally been used for its various medicinal properties. The current study was conducted to assess the comparative effect of *Murraya koenigii* (L.) *spreng* (curry leaves) and market available beta blocker drug Atenolol on cardiac enzyme (CK-MB) level in male albino rats. Out of total 26 locally bred male Albino Wistar rats (180 to 200gm weight) two rats were treated with only voltral for dose adjustment. Remaining 24 rats were randomly categorized into following 1 control (C) group and 3 experimental groups Model (M), Test 1 (T1) and Test 2 (T2) containing 6 rats in each group. Rats in C group were orally fed by 0.9% saline solution while rats of M and both test groups T1 and T2 were orally treated with voltral tablet (30mg /kg body weight) for three weeks to increase the level of CK-MB heart enzyme. After voltral treatment rats in test group T1 were treated orally with Atenolol (30 mg/kg body weight) and T2 with *Murraya koenigii* (L.) *spreng* (curry leaves) extract (180 mg/kg body weight) for last three weeks. Results show that rats treated with Atenolol showed a decrease in level of heart enzyme as compare to M group, while *Murraya koenigii* (L.) *spreng* treated rats group T2 showed more significant decrease of heart enzyme (CK-MB) level as compared to M and T1 groups with significantly improved behavioral activity including increased locomotor activity, short-term memory and reduction in depression. These results demonstrate that natural herbal treatment by curry leaves extract play an effective role in lowering the cardiac enzyme (CK-MB) level to its normal range which helps reducing the risk of CVD and CHD.

Keywords: *Murraya koenigii* (L.) spreng (curry leaves), atenolol, creatine kinase-muscle/brain level, cardiovascular diseases.

INTRODUCTION

Beta-blockers were traditionally recommended for the treatment of uncomplicated hypertension in ancient, but recent studies show outcomes of less than highest standard or quality with atenolol. (Elliott *et al*., 2011) (De Caterina *et al*., 2011). Atenolol is a hydrophilic cardio selective β-blocker, drug used in cardiovascular diseases including , angina, hypertensionacute myocardial infarction, supraventricular tachycardia, etc (Fox *et al*., 2007). Contrary lipophilic β-blockers, after myocardial infarction involve in critical arrhythmias and increases mortality and may not enhance remodeling of resistance arterioles in those with hypertension, compared with angiotensin-converting enzyme inhibitors (Maros *et al*., 2007). Atenolol along with other medicines treating congestive heart failure (ACE inhibitor, a diuretic and a digitalis-glycoside), decreases the utilization of oxygen of the heart muscle also reduces the muscular power of the heart so it start with low doses, which produces an undesired effect in congestive heart failure (Andrew *et al*., 2006.) It also slows conduction in the atrioventricular nose. Stroke index about 10% increases, usually it may decrease cardiac output by about 20% possibly secondary to its effect on heart rate. Myocardial contractibility and heart rate, as well as the reduction in blood pressure, produced by atenolol reported for the effectiveness of the drug in chronic stable angina pectoris. Similarly, it elevates oxygen supplies by increasing left ventricular fiber length and end-diastolic pressure, particularly in subjects with cardiac failure (McEvoy *et al*., 3003). Its high doses can cause side effects such as severe bradycardia, breathing problems, swollen ankles, tiredness, feeling faint, coldness in the arms or legs, (arrhythmia), headache, nightmares, (insomnia), (hallucinations), dryness of the mouth, eyes, (nausea), hair loss, skin rash, a serious allergic reaction to this drug (Ranjha *et al*., 2001). It inhibits sympathetic stimulation by binding at beta(1)-adrenergic receptors of the cardiac and smooth muscles that produces reduction in resting heart functions, cardiac output and negative chronotropic and inotropic activity respectively (Andrew *et al*., 2006). Medicinal herbs and bioactive compounds have been utilized in developing countries for primary and traditional health care system since many centuries. Voltral, a drug used to increase CK-MB consequently can

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produce side effects such as heart diseases. *Murraya koenigii (l.) Spreng* belongs to Rutaceae family commonly used as a flavouring agent in India has previously reported with significant medicinal properties such as antioxidant, antimicrobial, anticancer and prevention from renal damage. The antioxidants found in the curry leaves may produce effect in the elevation of (HDL-C) High density lipoprotein cholesterol and thus help to reduce the (LDL-C) low density lipoprotein cholesterol and TC. This antioxidant activity protects the oxidation of LDL, due to which an early event in the atheroma formation deactivated and hence keeps away from the coronary heart disease (CHD). Gajaria *et al* reported the protective activity of curry leaves have antioxidant potentials of flavonoids which oxidized LDL induced apoptosis in macrophages and reduce the production of unstable molecules from LDL oxidation (Molly *et al*., 2017). Thus, the aim of this study was to examine the significant outcome from curry leaves as compare to Beta blocker drug Atenolol on cardiac enzyme (CK-MB) level in reducing risk of cardiovascular events.

MATERIALS AND METHODS

Total twenty-six rats (bred male albino Wister) weighing about 180-200 grams were used throughout the study. The duration of study was between October, 2016 to January, 2017. Rats were purchased from research institute of Aga khan university, Karachi, Pakistan. Ethical approval of this study was obtained from ethical board of Federal Urdu University of arts, sciences and technology (FUUAST). Each rat was placed in separate cage with saw dust covered floor in quiet room with three axes, containing standard food and water at least 4 days before initiating experiment for incubation period to adopt themselves to new environment. Throughout the study period the temperature was maintained below 26°C and low relative humidity. The 12 hours light and dark cycle was maintained. The diet for rats contained all essential component such as carbohydrate, protein, amino acid and micronutrients.

Dose adjustment

For dose adjustment, trial study was done. Out of 26 rats, 2 rats were treated with voltral 10 mg/kg. This solution was given orally to rats. But only within 5 days of treatment one rat was died and other rat was decapitated has CK-MB 46 ng/ml, so the dose of drug was decreased to 6mg/kg body weight in other groups.

Experimental groups

Out of 26, remaining 24 rats were categorized into four groups. Each group consists of six rats. Group 1 called control treated with 0.9% normal saline. Other 3 groups called M, T1 and T2 were treated with voltral tablet 6mg /kg body weight for two weeks. At this range death of rats dd not occur, but it seemed that heart enzymes were increased. After voltral treatment group M remained same while T1 treated with Atenolol (30 mg/kg body weight) and T2 was treated with 8 curry leaves daily for 3 weeks.

Preparation of Voltral (10 mg)

Voltral (pulse 100mg) was purchased from local medical store and crushed than weigh 10mg. It was dissolved in 0.9% saline solution given orally to trial group of rats (Abdel-Daim *et al*., 2018).

Preparation of Voltral (6 mg)

Voltral (pulse 100mg) was also purchased from local medical store and crushed than weigh 6 mg. It was also dissolved in 0.9% saline solution given orally to rats M, T1, and T2 for three weeks. (Abdel-Daim *et al*., 2018).

Preparation of Atenolol (30 mg)

Atenolol (pulse 100mg) was purchased from local medical store and crushed than weigh 30mg. It was dissolved in 0.9% saline solution given orally to rat T1for last three week after voltral treatment. (Kamrun *et al*., 2018)

Preparation of curry leaves extract

Total 8 fresh curry leaves for a single rat were taken. It was crushed into paste form. It was than dissolved in 0.9 % saline solutions and was given to T2 rats orally for last 3 weeks after voltral treatment.

Behavioral technique

Behavioral activity was done in quiet room without noise effect, under normal day light of all rats were noted for 1^{st}, 3^{rd} and 6^{th} week of treatment to observe the effect of drug on animal.

Open field activity

In open field apparatus, activity was monitored for 10 minutes. This apparatus was usually used for qualitative and quantitative measures of general locomotor activity, willingness and marked with a grid and square crossing. It consists of 36 cm high wall with a square area (72 × 72 cm). The lines divided the floor into sixteen 18 x 18 cm squares. The central square is used because some rats strains have high locomotor activity and cross the lines of the test chamber An animal was placed in center square and its movement, crossing with all four paws, corner sitting, grooming and gnawing activities were recorded.

Light and dark apparatus

Light and dark apparatus was used for anxiety. It consists of small square area (26x26x26) with an access (12x12 cm) walls with one compartment transparent and second were entered on the dark side and time takes to rat move in the light portion within 5 minutes was noted.

Home cage activity

Specially designed Perspex home cage (26x26x26 cm) with saw dust covered floor was used to monitor activities

as the number of cages crossing and 0.4 scales of increasing intensities of grooming and gnawing.

Forced swimming activity

The behavioral despair test also known as forced swimming test used for rodent's response to the threat of drowning and to measure the effectiveness of antidepressant. These results have been interpreted as negative mood. In this model two trials are done in which animals are forced to swim in an acrylic glass cylinder filled with water, and from where rats cannot escape. First trial lasts for 15 minutes, then after 24-hours, a second trial was performed for 5 minutes. In second trial, time that rat spends without making any movement and keep its head above water was measured. Because, various types of antidepressants and electroconvulsive shock decreases the immobility time.

Water maze activity

In water maze activity, rats were placed into the water filled tank first for training session and allowed 120 seconds to locate and climbed into the submerged platform. Platform is hide by the milk so that the rats cannot easily find the platform. For 15 seconds rats were allowed to stay on this platform. If the rats did not find the platform within time then they were guided gently onto the platform. After training session short term and long term memory was noted for each rat.

Tissue extract preparation

Rats were killed by dislocating neck from base of the skull. Tissues including brain, heart, and liver were taken out and washed in ice cold 0.9% saline then stored in the formalin.

Analysis of heart enzyme

Only Heart enzyme (CK-MB) was analyzed by blood specimen using spectrophotometric method. The test was performed in DOW University Ojha campus Karachi-Pakistan, using kit method (Huaguo *et al*., 2015)

STATISTICAL ANALYSIS

Statistical analysis was done using statistical package for social science (SPSS Version. 20). Results presented in terms of mean ± SD (n=6). Significant dissimilarity calculated by Tukey HSD and Duncan test P value ($p<0.05$) following one-way ANOVA, considered significant. Significant difference was determined in T1and T2 as compare to M and C group.

RESULTS

Behavioral result

In fig. 1(a) open filed activity statistically analyzed by one-way ANOVA (df 3.23) ($F=112.8063$) ($P<0.003$) results how that after 6 weeks treatment, locomotor activity of T1 and T2 rats was increased but, T2 rats show the significantly increase in locomotor activity as compare to T1 and M group. fig. 1(b) Home cage activity statistically analyzed by one-way ANOVA (df 3.23) ($F=112.8063$) ($P<0.002$) show that after 6-weeks treatment the stimulatory activity of T1 group was decreased as compare to M but T2 group show significant increase in stimulatory activity as compare to T1 and M group. fig. 1(c) Force swimming test was Statically analyzed by one-way ANOVA (df 6, 23) ($F=90.043$) ($p<0.004$) determined that after treatment of 6 weeks depression was decreased in T1 and T2 group as compare to M group. fig. 1(d) water maze activity (short term memory) was statistically analyzed by one-way ANOVA (df 3.23) ($F=89.903$) ($P<0.002$) was statistically significant show that after 6-weeks treatment recognition memory in T2 group was markably high as compare to T1 and M group. Fig. 1(e) Water maze activity (long term memory) statically analyzed by one-way ANOVA (df 6, 23) ($F=113.2$) ($p<0.03$) in both T1 and T2 group of rats was markedly increased as compare to M group after 6 weeks of treatment.

Cardiac enzymes

Fig. 2(a) statically analyzed by one-way ANOVA (df 6, 23) ($F=981.415$) ($p<0.03$) show the effect of voltral on heart enzyme CK-MB. Results for M, T1 and T2 indicate the higher enzyme activity after treatment of voltral as compare to C group. Higher level of CK-MB suggested that there is disease or damage to the heart. Fig. 2(b) statically analyzed by one-way ANOVA (df6, 23) ($F=168.04$) ($p<0.001$) show the effect of atenolol and curry leaves after treatment. In group T1 treated with atenolol show decreased level of heart enzyme as compare to M group, while group T2 treated with curry leaves show more significant decrease of heart enzyme level as compared to M and T1 groups.

DISCUSSION

Atenolol is well known beta-blocker and effective for the treatment of hypertension arrhythmias and coronary heart disease (Berthonneche *et al*., 2009). In our study *Murraya Koeinigii (L.) Spreng* showed the more significant decrease in cardiac enzyme (CK-MB) level to its normal range and produced significantly improved effect on behavioral activities as compared to drug (atenolol) treated rats. Voltral is used in our study to increase the heart enzymes, as only cardiac enzyme (CK-MB) was diagnosed in our study before and after treatment (Abdel-Daim *et al*., 2018). It was supposed that *Murraya Koeinigii* (L.) *Spreng* has an active compound similar to atenolol that produce a positive effect on heart by decreasing cardiac enzyme. Behavioral activity results compared between treated groups with the control and model groups fig. 1(a) revealed locomotors activity in open field apparatus made of wood to check movements.

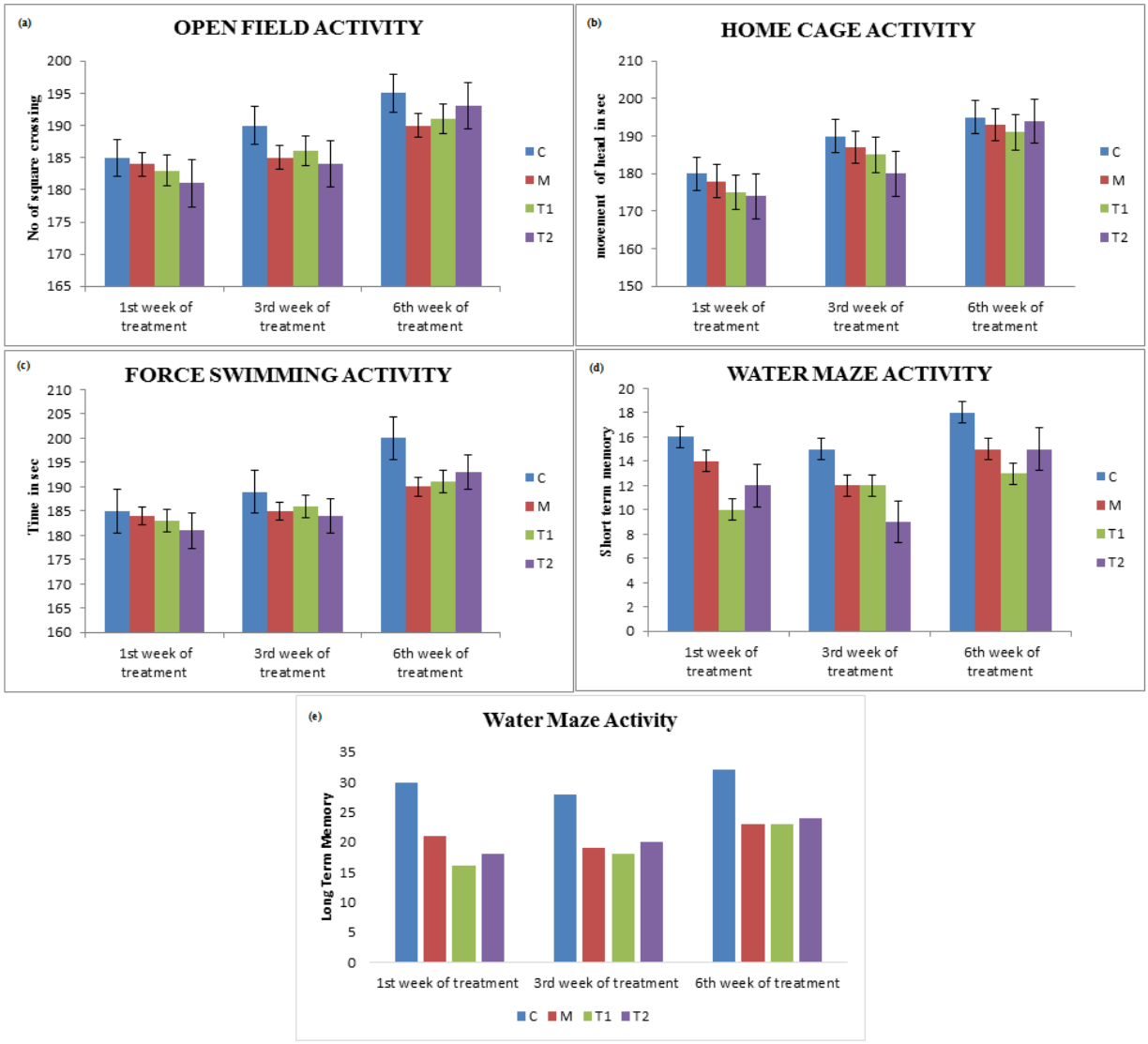

Fig. 1: Comparison of Behavioral results in Control (C), Model (M), Test 1 (T1) and Test 2 (T2) groups.

Cerebrum region of brain involve in the movement, senses as well as thoughts. Group of rats treated with herbal extract show increase no. of crossing as compare to group treated with drug which reveals curry leaves enhance locomotor activity. The rats treated with curry leaves showed significant stimulatory activity as compared to others groups. For monitoring of depression in rats forced swimming activity was performed and group of rats treated with drug showed increased depression than model and curry leaves treated rats. It means herbal extract such as curry leaves also exhibits antidepressant activity. Similarly, short term and long-term activity was also observed through water maze activity. Group of rats treated with curry leaves showed significant increase in last week of observation. Whereas drug treated rats show increase in long term memory as compared to other groups.

The increased level of enzyme creatinine kinase, specifically CK-MB, produces heart attack within hours as the heart muscle cells die (McEvoy *et al*., 2003). In the previous study, isoproterenol (ISO) administration to animal model rats caused elevations in activities of the serum cardiac enzyme activities. But CK-MB activity in the serum is considered as sensitive and significant diagnostic marker of myocardial infaraction (MI). Pretreatment with honey significantly decreases the elevated levels of serum cardiac marker enzymes, it describe Tualang Honey (TH) restricts the leakage of these cardiac enzymes (Ibrahim *et al*., 2015). Similarly, to our study a recent study suggested that antioxidant property of curry leaves prevent coronary heart disease (CHD) and cardiovascular disease (CVD) (John Molly *et al*., 2017). On the other hand, this study animal model with increased cardiac enzyme show that by administration of curry leaves extract significant

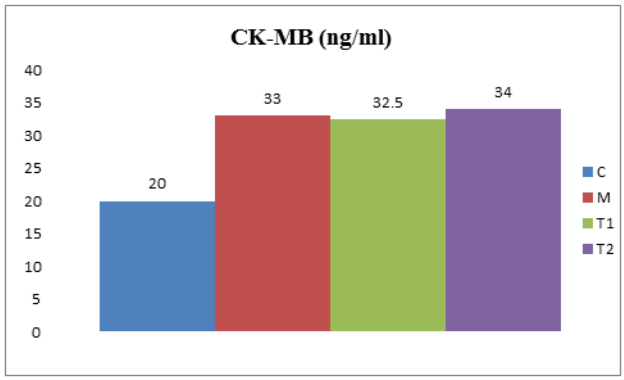

Fig. 2(a): Effect of heart enzyme (CK-MB) after treatment of voltral

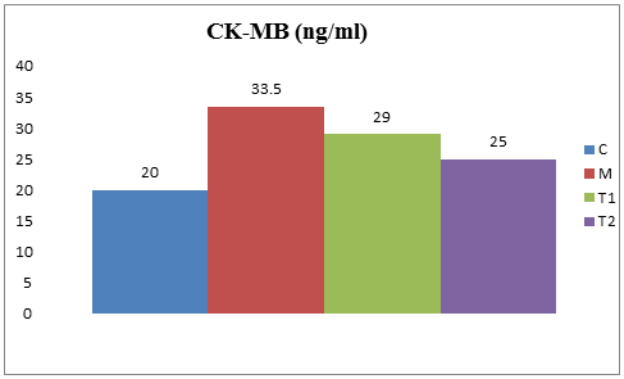

Fig. 2(b): Effect of heart enzyme (CK-MB) after atenolol and *Murraya Koeinigii (L.) Spreng* (curry leaves)

Table 1: Mean ± S.D (Standard Deviation) of Heart enzyme Test compared between Control (C), Model treated (M), Atenolol (T1) and *Murraya Koeinigii (L.) Spreng* (T2)

Heart Enzyme Test	Mean ± S.D of Control (C)	Mean ± S.D of Model (M)	Mean ± S.D of Test (T1) Atenolol treated	Mean ± S.D of Test (T2) *Murraya Koeinigii* (L.) *Spreng* treated	f-value
CK-MB (After voltral treatment)	± 3.14	33 ± 2.56	32.5 ± 2.93	*34 3.79	5.6132
CK-MB (After Atenolol and *Murraya Koeinigii* (L.) *Spreng*)	20 ± 4.13	33.5± 3.21	29 ± 3.01	*25 ± 2.45	6.01293

*show result is significant

reduction in the CK-MB was observed. These results indicate that curry leave extract decreases the incidence of CHD or CVD.

CONCLUSION

The result of present study concludes the consumption of curry leaves extract is more effective in lowering the levels of heart enzyme (CK-MB) and due to its hypolipidemic effects, it prevents from complications such as coronary heart disease and cardiovascular disease. In addition it also exhibit anti-depressive effect with improved behavioral activities. It may also have lesser side effect as compared to drug atenolol given in cardiac diseases. Although, further large perspective studies are still required in this regard.

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