Study of Methanolic Extract of *Artemisia pallens* Wall on Endurance of Laboratory Animals

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**ABSTRACT**

**Background:** Various anabolic steroids are used to improve endurance and muscle strength. The use of such synthetic drugs is banned by World Anti-Doping Agency due to their adverse and toxic effects. On the other hand, traditional Indian medicinal plants were used by the athletes to improve their endurance and muscle strength. The objective of this study was to evaluate the effect of methanolic extract of *Artemisia pallens* Wall on endurance of laboratory animals.

**Materials and methods:** Endurance in male Wistar rats (150-180 g) was achieved by treadmill running exercise. The animals were trained for two weeks. The Methanolic Extract of *Artemisia pallens* Wall (MEAP) was administered orally to the animals for the next 6 weeks and parameters such as CKMB, BUN, serum creatinine, serum glucose, testosterone, total protein, complete lipid profile, heart weight to body weight ratio, electrocardiographic and hemodynamic were evaluated. Lung histopathological assessments were performed. **Results:** Eight weeks administration of MEAP (100 and 200 mg kg⁻¹) significantly (p<0.05) lowered the serum cholesterol and HDL levels, whereas the serum LDL and triglyceride levels were increased significantly (p<0.05). There was significant (p<0.05) decrease in serum CKMB and creatinine by MEAP (100 and 200 mg kg⁻¹) treatment. MEAP (100 and 200 mg kg⁻¹) was significantly (p<0.05) inhibited breakdown of protein to urea. The electrocardiographic changes like prolongation in QT and QTc intervals were seen with the MEAP (100, 200 and 400 mg kg⁻¹) treatment. Histological aberrations were also ameliorated by MEAP treatment. **Conclusion:** MEAP (200 mg kg⁻¹) significantly improved the endurance and performance of the animals.

**Key words:** *Artemisia pallens*, blood pressure, creatinine, endurance, testosterone

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**INTRODUCTION**

Endurance training is the act of exercising to increase stamina and endurance. The term ‘endurance training’ generally refers to training the aerobic system as opposed to anaerobic (Devi et al., 2003). The need for endurance in sports is often predicated as the need of cardiovascular and simple muscular endurance. Endurance training is essential for a variety of endurance sports. A notable example is distance running events (800 meters up to marathon) with the required degree of endurance training increasing with race distance (Fenning et al., 2003; Wisloff et al., 2001).

Before the advent of synthetic drugs, sportsmen used herbs to enhance physical performance. Recent interest in the use of herbs like ginseng from various countries and ephedra (Lieberman, 2001), *Withania somnifera* (Grandhi et al., 1994), *Rhodiola rosea* (De Bock et al., 2004), yohimbine, *Corynephorus* fungus, (Ghosal et al., 1988) and many more have been reported to enhance physical performance. Improvement in muscular strength, maximal oxygen uptake, work capacity, fuel homeostasis, serum lactate, heart rate, visual and auditory reaction times, alertness and psychomotor skills have been documented with Asian ginsengs (Bucci, 2000). Siberian ginseng has shown mixed reactions, combinations with caffeine are required for Mahuang, ephedrine and related alkaloids to show beneficial effects on physical performance (Bucci, 2000). Other herbs remain virtually unexplored. In this context, it is necessary to develop animal models for evaluation of ergogenic actions of herbs for physical performance (Kandhare et al., 2011b).
Artemisia pallens Walls ex D.C. (Davana) (Asteraceae) is an aromatic medicinal herb native in the southern part of India, especially in the states of Karnataka, Tamil Nadu andhra Pradesh and Maharashtra. Chemical composition of oil obtained from A. pallens plant has been investigated by number of researcher (Sipma and Van der Wal, 1968; Rojatkar et al., 1996; Pujar et al., 2000). The oil of A. pallens is used as flavouring agent for cakes, pastries, tobacco and in some costly beverages. Artemisia pallens possesses anthelminthic, tonic, antipyretic, antidiabetic, antifungal, antibacterial, antimicrobial, antioxidant, analgesic and anti-inflammatory activity (Ashok and Upadhyaya, 2010; Ruikar et al., 2011). There is paucity of research studies on the plant which can have beneficial effects in sports personnel without attracting the stigma of doping. The objective of the study was to evaluate the effect of methanolic extract of aerial part of Artemisia pallens on endurance in wistar rats.

MATERIALS AND METHODS

Animals: Adult male Wistar rats (150-180 g) were procured from National Toxicology Centre, Pune, India. The rats were housed at 24±1°C, with relative humidity of 45-55% and 12:12 h dark/light cycle. The animals had free access to standard pellet chow (Pranav Agro industries Ltd., Sangli, India) and water ad libitum. All experiments were carried out between 09:00 and 17:00 h. The experimental protocol was approved by the Institutional Animal Ethics Committee (IAEC) of Poona College of Pharmacy, Pune and performed in accordance with the guidelines of Committee for Control and Supervision of Experimentation on Animals (CPCSEA), Government of India on animal experimentation.

Drugs and chemicals: Deca-durabolin was obtained from Candila Healthcare Ltd., Goa, India. 1,1,3,3′-Tetraethoxypropane, crystalline beef liver catalase, reduced glutathione (GSH), 5, 5′-dithiobis (2-nitrobenzoic acid) were purchased from S.D. Fine chemicals, Mumbai, India. Sulphanilamides, naphthalamine, diamine HCl and phosphoric acid were obtained from LobaChemie Pvt. Ltd., Mumbai, India.

Preparation of extract Artemisia pallens: The aerial parts of Artemisia pallens Wall were collected from Jejuri, Maharashtra; botanically identified and authenticated by Dr. A.S. Upadhye, Scientist, Plant Drug Authentication Service, Agharkar Research Institute, Pune, India. The voucher specimen of plant material was maintained under the reference number WP-991. The aerial parts of the plant were shade dried and size reduced to obtain a coarse powder. The weighed quantity (500 g) of powder was cold macerated with 7.5 L of methanol (99.5% v/v) for one week with occasional shaking. The macerate was then filtered and the filtrate was evaporated under reduced pressure using vacuum evaporator (Rotavac, Equitron) to obtain a dark green colored extract.

Experimental design: Male Wistar rats were randomly divided into six groups of 6 rats as follows:

Group 1: Normal Control (NC): Rats did not receive any training and were administered single daily dose of 10 mg kg⁻¹ of distilled water, p.o. for 8 weeks
Group 2: Exercise Control (EC): Rats received training and were administered single daily dose of 10 mg kg⁻¹ of distilled water, p.o. for 8 weeks
Group 3: Deca-durabolin (DDB) (5 mg kg⁻¹): Rats received training and administered with deca-durabolin (5, subcutaneously, twice a week)
Group 4: MEAP (100 mg kg⁻¹): Rats received training and were administered single daily dose of 100 mg kg⁻¹ of methanolic extract of Artemisia pallens
Group 5: MEAP (200 mg kg⁻¹): Rats received training and were administered single daily dose of 200 mg kg⁻¹ of 1% aqueous solution of Artemisia pallens
Group 6: MEAP (400 mg kg⁻¹): Rats received training and rats were received training and were administered single daily dose of 400 mg kg⁻¹ of methanolic extract of Artemisia pallens

Rats were trained over treadmill apparatus for 2 weeks prior to the treatment to minimize the stress. The drug treatment was given after 2nd week and continued till 8th week. Training protocol was followed during the entire study for the development of endurance. On the final day animals were made to run till exhaustion. Biochemical and ECG parameters were carried out on day 0, 2nd week and 8th week. Hemodynamic parameters were carried out 24 h after exhaustion (failure of rat to move onto the running treadmill belt after receiving electric shock three consecutive times).

Training protocol: Each rat ran on a treadmill at a low initial speed followed by gradual increase in running speed and time until the point of exhaustion. The rats were made to run for 5 days a week:

• In the 1st week, 10 m min⁻¹ for 10 min at 0° elevation was maintained on 1st and 2nd days, 15 m min⁻¹ for 10 min at 0° elevation was maintained on 3rd, 4th and 5th days
• In the 2nd week 19 m min⁻¹ for 15 min at 0⁰ elevation was maintained for the entire week
• In the 3rd week 20 m min⁻¹ for 20 min at 0⁰ elevation was maintained for the entire week
• In the 5th week 15 m min⁻¹ for 30 min at 5⁰ elevation was maintained for the entire week
• In the 6th week 15 m min⁻¹ for 40 min at 5⁰ elevation was maintained for the entire week
• In the 7th week 15 m min⁻¹ for 50 min at 5⁰ elevation was maintained for the entire week
• In the 8th week 15 m min⁻¹ for 60 min at 5⁰ elevation was maintained for the entire week

**Estimation of biochemical parameters:** On 2nd and 8th week of the study, rats were anaesthetized with anesthetic ether. Blood was drawn from the retro-orbital plexus and serum was separated by centrifugation using Eppendorf Cryocentrifuage (model No, 5810, Germany), maintained at 4°C and run at speed of 7000 rpm for 15 min. Blood sugar level, creatinine, total protein, cholesterol, triglycerides, HDL, LDL and BUN were measured by using standard kits (Accurex Biomedical Pvt. Ltd., Mumbai, India) according to earlier reported methods (Kamble et al., 2013; Kandhare et al., 2011a; Kandhare et al., 2013; Visnagri et al., 2012, 2014; Kumar et al., 2013).

**Assessment of electrocardiographic changes:** ECG was recorded under mild ethereal anaesthesia by placing the leads on the right foreleg (negative electrode), left foreleg (positive electrode) and right hind leg (neutral electrode) of the rats. Electrocardiographic changes were recorded using an eight-channel Power Lab System (LabChart 7.3; AD Instrument Pvt. Ltd.) according to earlier reported methods (Visnagri et al., 2013).

**Assessment of hemodynamic changes:** Then animals were anaesthetized by urethane (1.25 g kg⁻¹, i.p.). Blood pressure was measured using a polyethylene cannula (PE 50) filled with heparinized saline (100 IU mL⁻¹), inserted into the left carotid artery. The cannula was connected to a transducer and the signal was amplified by a bioamplifier. Left ventricular systolic pressure was measured using a Millar micro-tip transducer catheter (Model SRP-320; Millar Instrument Inc., Houston, Texas, USA) inserted into the left ventricle through the right carotid artery and connected to a bioamplifier. Heart rate, dP/dt max, dP/dt min and left ventricular end-diastolic pressure signals were obtained from primary signals (left ventricular systolic pressure and blood pressure) using a data acquisition system (LabChart 7.3; AD Instrument Pvt. Ltd).

**Histopathological examination of heart tissue:** Heart tissues were stored in 10% formalin for 24 h. The specimen was dehydrated and placed in xylene for 1 h (3 times) and later in ethyl alcohol (70, 90 and 100%) for 2 h, respectively. The infiltration was carried out by treating with paraffin wax twice, each time for 1 h. Tissue specimens were cut into sections of 3-5 μm thickness and were stained with hematoxylin and eosin (H and E). The specimens were mounted on slide by use of Distrene Phthalate Xylene (DPX) as mounting medium. Sections were examined under a light microscope for inspection of the histopathology features of specimen and infiltration of cells.

**Statistical analysis:** Results are expressed as Mean±SEM. Data analysis was performed using GraphPad Prism 5.0 software. Statistical comparisons were made between drug-treated groups and exercise control animals. Biochemical parameters were statistically analyzed using two way ANOVA followed by Bonferroni’s post-hoc test while data of ECG and hemodynamic parameters was analyzed using one-way ANOVA followed by Bonferroni’s multiple comparison test for post hoc analysis. A value of p<0.05 was considered to be statistically significant.

**RESULTS**

Treatment with MEAP significantly (p<0.05) reduced the body weight and blood sugar levels of the animals when compared to the exercise control animals (Fig. 1a, b). Serum total protein level was significantly (p<0.05) reduced in MEAP (200 mg kg⁻¹) whereas, with MEAP (100 and 400 mg kg⁻¹) the reduction was non-significant when compared to exercise control group (Fig. 1c). The serum testosteone levels were markedly elevated (p<0.05) with the higher doses of MEAP (200 and 400 mg kg⁻¹) whereas treatment with deca-durabolin and MEAP (100 mg kg⁻¹) significantly (p<0.05) reduced the levels compared to exercise control group (Fig. 1d).

Treatment with MEAP caused a significant (p<0.05) decrease in serum CKMB levels in a dose dependent manner when compared to exercise control group (Fig. 2a). Administration of MEAP significantly (p<0.05) reduced the serum creatinine levels when compared to exercise group (Fig. 2b). The BUN levels were non-significantly reduced on treatment with MEAP (Fig. 2c).

MEAP treatment showed significant (p<0.05) reduction in serum cholesterol (Fig. 3a), serum HDL (Fig. 3c) and serum LDL levels (Fig. 3d), whereas the opposite effect was seen in case of serum triglyceride levels (Fig. 3b) compared to exercise control group. MEAP (200 mg kg⁻¹) caused marked decrease in serum
Fig. 1(a-d): Effect of MEAP treatment on (a) Body weight, (b) Blood sugar level, (c) Serum total protein and (d) Serum testosterone levels of rats. Data are expressed as Mean±SEM and analyzed by Two way ANOVA followed by Bonferroni's post-hoc test. *p<0.05 as compared with the exercise control group, **p<0.01 as compared to normal control group and ***p<0.001 as compared to DDB group. NC: Normal control group, EC: Exercise control group, DDB (5): Deca-durabolin (5 mg kg⁻¹, s.c.) treated group, MEAP (100): Methanolic extract of Artemisia pallens (100 mg kg⁻¹, p.o.) treated group; MEAP (200): Methanolic extract of Artemisia pallens (200 mg kg⁻¹, p.o.) treated group and MEAP (400): Methanolic extract of Artemisia pallens (400 mg kg⁻¹, p.o.) treated group.

LDL levels, on the other hand, this effect was not observed in case of the other two doses (Fig. 3).

At the end of the study the Heart Weight to Body Weight ratio (HW/BW) which was high in exercise group was significantly (p<0.05) reduced by the treatment of MEAP (Table 1). MEAP (100, 200 and 400 mg kg⁻¹) significantly (p<0.05) increased the heart rate when compared to exercise control group (Table 1). The QRS interval was significantly (p<0.05) reduced with MEAP (100 mg kg⁻¹) whereas, the other two doses caused prolongation in QRS interval. The QT (p<0.05) and QTc intervals were reduced on treatment with MEAP (200 mg kg⁻¹) on the other hand this was not seen with the other two doses. There was significant (p<0.05) prolongation of P duration with MEAP (100 and 400 mg kg⁻¹) but the effect was non-significant in case of treatment with MEAP (200 mg kg⁻¹) when compared with exercise control group (Table 1). Systolic and diastolic blood pressures were significantly (p<0.05) reduced with the treatment of MEAP compared to exercise control group. There was a significant elevation in max dp/dt (p<0.05) and an opposite effect was seen in case of Min dp/dt (p<0.05) on treatment with MEAP when compared to exercise control group (Table 2).

Histological assessment of heart tissue from exercise control animals showed normal architecture. There was no evidence of cardiomyocyte hypertrophy, nucleomegaly or cytoplasmic acidophilia. There was no evidence of inflammation, degenerative changes or necrosis (Fig. 4a). Histological examination of myocardial tissue of the exercise control group showed normal architecture. There was no evidence of cardiomyocyte hypertrophy, nucleomegaly or cytoplasmic acidophilia. There was no evidence of inflammation, degenerative changes or necrosis (Fig. 4b). Histological examination of myocardial tissue of the deca-durabolin (10 mg kg⁻¹ week) group showed normal architecture with mild subendocardial inflammation. There was no evidence of cardiomyocyte hypertrophy, nucleomegaly or cytoplasmic acidophilia. There was no evidence of inflammation, degenerative changes or necrosis (Fig. 4c). Histological examination of
Fig. 2(a-c): Effect of MEAP treatment on (a) Serum creatine kinase-MB (b) Serum creatinine and (c) Blood urea nitrogen levels of rats. Data are expressed as Mean±SEM and analyzed by Two way ANOVA followed by Bonferroni’s post-hoc test. *p<0.05 as compared with the exercise control group, †p<0.05 as compared to normal control group and ‡p<0.05 as compared to DDB group. NC: Normal control group, EC: Exercise control group, DDB (5): Deca-durabolin (5 mg kg⁻¹, s.c.) treated group, MEAP (100): Methanolic extract of *Arenisca pallens* (100 mg kg⁻¹, p.o.) treated group; MEAP (200): Methanolic extract of *Arenisca pallens* (200 mg kg⁻¹, p.o.) treated group and MEAP (400): Methanolic extract of *Arenisca pallens* (400 mg kg⁻¹, p.o.) treated group

Fig. 3(a-d): Continue
Fig. 3(a-d): Effect of MEAP treatment on (a) Serum cholesterol, (b) Serum triglyceride, (c) Serum high density lipoprotein (HDL) and (d) Serum low density lipoprotein (LDL) levels of rats. Data are expressed as Mean±SEM and analyzed by Two way ANOVA followed by Bonferroni's post-hoc test. *p<0.05 as compared with the exercise control group, †p<0.05 as compared to normal control group and ‡p<0.05 as compared to DDB group. NC: Normal control group, EC: Exercise control group, DDB (5): Deca-durabolin (5 mg kg⁻¹, s.c.) treated group, MEAP (100): Methanolic extract of Artemisia pallens (100 mg kg⁻¹, p.o.) treated group; MEAP (200): Methanolic extract of Artemisia pallens (200 mg kg⁻¹, p.o.) treated group and MEAP (400): Methanolic extract of Artemisia pallens (400 mg kg⁻¹, p.o.) treated group.

Fig. 4(a-f): Effect of MEAP treatment on pathological alteration in rat heart. Photomicrograph of sections of heart of (a) Normal control rat, (b) Exercise control rat (c), Deca durabolin (5 mg kg⁻¹, s.c.) treated rat, (d) MEAP (100 mg kg⁻¹, p.o.) treated rat (e), MEAP (200 mg kg⁻¹, p.o.) treated rat and (f) MEAP (100 mg kg⁻¹, p.o.) treated rats. H and E staining at 40X

myocardial tissue of the MEAP (100 mg kg⁻¹) group showed normal architecture. There was no evidence of cardiomyocyte hypertrophy, nucleomegaly or cytoplasmic acidophilia. There was no evidence of inflammation, degenerative changes or necrosis (Fig. 4d). Histological examination of myocardial tissue of the MEAP (200 mg kg⁻¹) group showed normal architecture. There was no evidence of cardiomyocyte hypertrophy,
nucleomegaly or cytoplasmic acidophilia. There was no evidence of inflammation, degenerative changes or necrosis (Fig. 4c). Histological examination of myocardial tissue of the MEAP (400 mg kg⁻¹) group showed normal architecture. There was no evidence of cardiomyocyte hypertrophy, nucleomegaly or cytoplasmic acidophilia. There was no evidence of inflammation, degenerative changes or necrosis (Fig. 4d).

**DISCUSSION**

In the present study deca-durabolin was used as an anabolic steroid. Anabolic steroids are synthetic derivatives of testosterone used in therapeutic doses in medical practice. In addition high doses of anabolic steroids have been used by athletes to improve physical performance. In athlete's anabolic steroids increases protein metabolism and consequently strength, potency and muscle mass. Anabolic steroids promote myocardial structural changes. Previous studies have suggested that high doses of anabolic steroids associated with exercise training resulted in impaired lipid and lipoprotein metabolism, increased atherosclerosis, abnormal blood coagulation (Cunha et al., 2005; Nieminen et al., 1996), moderate cardiac hypertrophy with adequate cardiac capillarization (Tagrakis et al., 2000), increase in arterial blood pressure (Kuipers et al., 1991), apoptotic cell death of ventricular myocyte in vitro (Zaugg et al., 2001) and reduction in cardiac contractility in animals (Trifunovic et al., 1995) and athletes (Urhausen et al., 1989). Rocha et al. (2007) reported that exercise training associated with anabolic steroids induces maladaptive remodeling and future deterioration in cardiac performance (Rocha et al., 2007). Exercise training associated with anabolic steroids cause loss of the beneficial effects in left ventricular function induced by exercising. Anabolic steroid plus exercise increases cardiac collagen content associated with activation of local renin-angiotensin. High doses of anabolic steroids have adverse effects on the hepatic, endocrine and cardiovascular systems.

In the present study MEAP showed some distinct similarities and differences on the biochemical markers as well as hemodynamic parameters when administered orally in rats trained to undergo strenuous exercise as running on the moving belt of treadmill.

It has been reported that anabolic steroids decrease the body weight (Rocha et al., 2007). The probable reason for this effect is associated with decrease in the intraperitoneal fat. Deca-durabolin and MEAP significantly reduced the body weight after exercise indicating utilization of intraperitoneal fat for production of energy required for exercise.

Increased utilization of blood sugar transporting it into the muscle is essential for providing energy to the muscles during exercise (Ghosh et al., 2012; Kandhare et al., 2012). Treatment with deca-durabolin and MEAP showed significant reduction in blood sugar level compared to that of exercise control. *Artemisia pallens* (methanol and acetone extracts of aerial parts) has been reported to possess dose dependent and significant reduction in blood glucose level (Subramoniam et al., 1996). The effect of the extract on the blood sugar of exercise trained animals has not been reported. The possibility of having difference in the potency of lowering blood glucose in normal rats compared to diabetic rats cannot be ruled out.
Earlier reports have indicated that chronic administration of deca-durabolin (s.c.) twice a week 10 mg kg\(^{-1}\) week produced significant increase in serum testosterone concentration (Rocha et al., 2007). Our results showed decrease in serum testosterone compared to that of exercise control. The exogenously administered steroids suppress the hypothalamic-pituitary-gonadal axis, which acts as a feedback system and disturbs the endogenous production of testosterone and gonadotropins (Hickson et al., 1994). These findings corroborate with our results since deca-durabolin reduced the serum testosterone level. The exogenous testosterone probably was able to cause a negative feedback in response to gonadotropins and was thus responsible for the observed drop in circulating serum testosterone levels. MEAP treatment showed a paradoxical effect where as MEAP at higher concentrations of 200 and 400 mg kg\(^{-1}\) showed significant elevation in serum testosterone levels. This effect needs further investigations as the extract may contain some unknown ingredients which may influence the hypothalamic-pituitary-gonadal axis and the feedback system.

It has been reported that deca-durabolin presents an anabolic activity (Katz and Pope, 1990). Most of the drugs bind to the receptors in the muscle decreasing the circulating portion. Decrease in serum total protein levels by deca-durabolin in the present study indicated that the proteins may be utilized in muscle building, thereby decreasing the serum total protein. However, MEAP treatment showed paradoxical results wherein at 200 mg kg\(^{-1}\) decrease in serum total protein was observed, whereas the opposite effects of increase in the serum total protein was observed with 100 and 400 mg kg\(^{-1}\) concentrations. However when compared with serum total protein of exercise control animals the increase was non-significant. In this respect MEAP differs from that of deca-durabolin.

Serum creatinine, blood urea nitrogen are regarded as important parameters of kidney function test (Visnagri et al., 2012). Creatinine is an endogenous amine produced as a result of muscle catabolism. It is excreted unchanged in the urine by glomerular filtration only. In the exercise control group a significant increase in serum creatinine level compared to control group indicated increased catabolism. However deca-durabolin and MEAP reduced serum creatinine level indicating reduction in catabolism. MEAP (200 mg kg\(^{-1}\)) appears to be equivalent with deca-durabolin in reducing the catabolism. Accumulation of blood urea nitrogen indicated increase in the urea which is an end product of protein catabolism. In the exercise control group after 8 weeks, marginal increase in blood urea nitrogen was observed and in the treatment groups the increase was non-significant. The results clearly indicated that the breakdown of protein to urea is arrested by the treatment.

Several diagnostic marker enzymes like creatine kinase-mb isoenzyme and lactate dehydrogenase present in the myocardium are used as a predictor for pathological changes in heart (Visnagri et al., 2013). These enzymes are released into the extracellular fluid during myocardial injury (Balazs and Ferrans, 1978). Creatine kinase-mb is a standard marker of myocardial injury or death. Creatine kinase-mb leaks out from the myocardium due to disintegration of contractile apparatus and increased sarcoplasmic permeability (Mair et al., 1994). Increase in the serum creatine kinase-mb indicates myocyte injury. In the exercise control animals after training decrease in the serum creatine kinase-mb indicated lack of detrimental effect of exercise on the heart. Deca-durabolin as well as MEAP (100-400 mg kg\(^{-1}\)) did not increase serum creatine kinase-mb and decrease in serum creatine kinase-mb was observed which confirms the protective effect of test substances on the heart.

In clinical study carried out by previous researcher showed reduction in the levels of LDL, total cholesterol, HDL and triglycerides in individuals who have undergone resistance training and made use of steroid (Venacio et al., 2008). Another study reported decrease in HDL after use of anabolic steroids (Dobs et al., 2001). Deca-durabolin and MEAP reduced significantly serum cholesterol and HDL. Deca-durabolin and MEAP showed opposite effects on serum LDL. Deca-durabolin decreased serum LDL whereas MEAP increased serum LDL non-significantly compared to that of exercise control. A dose dependent effect of MEAP was not observed. Triglyceride levels were significantly reduced in exercise control compared to the control but deca-durabolin and MEAP increased the triglyceride levels compared to the exercise control.

Heart weight to body weight ratio showed a non-significant increase in the exercise control animals compared to that of control animals. Endurance training is known to cause eccentric cardiac hypertrophy in which adaptive responses are distributed across the left ventricular wall. However, many factors influence exercise induced cardiac hypertrophy. Some of them are the mode, intensity, duration and the frequency of exercise regimen (Evangelista et al., 2003). It has been reported that duration controlled swimming exercise training induces cardiac hypertrophy in mice (Evangelista et al., 2003). In the present study increase in the heart weight and increase in the body weight of exercise control animals was observed which is reflected in heart weight to body weight ratio. The heart of the exercise control animals was larger in size compared to
the control animals. Treatment with deca-durabolin and MEAP reduced the heart weight to body weight ratio. This effect was an outcome of maintaining the heart weight and decrease in body weight in the treatment groups. The results clearly indicated that the cardiac hypertrophy produced by the endurance training is effectively reduced by the treatment.

The heart rate in the rodents is higher than the human beings. The heart rate of rats in the earlier study was in the range of 300 to 322 bpm (Zarwar et al., 2011). The heart rate of control animals that is 325.67 bpm correlates with the heart rate reported by other workers in our laboratory (Zarwar et al., 2011). Endurance training resulted in significant decrease in the heart rate; the resulting resting bradycardia confirms effectiveness of the exercise training. Deca-durabolin and MEAP treatment showed increase in the heart rate and reversed the bradycardia produced by the endurance training. However a dose response effect in case of MEAP could not be established as MEAP (200 mg kg\(^{-1}\)) was weak in reversing the resting bradycardia.

In the present investigation endurance training did not increase QRS interval compared to control. Deca-durabolin treatment significantly increased QRS interval compared to exercise control indicating impairment of sodium influx. MEAP (100 mg kg\(^{-1}\)) treatment showed non-significant decrease compared to exercise control which indicates that at this dose the extract did not impair influx of sodium. The result of MEAP (200 mg kg\(^{-1}\)) appears to be paradoxical as increase in QRS was observed similar to that of deca-durabolin. However the highest dose of MEAP (400 mg kg\(^{-1}\)) did not increase QRS interval compared to control.

The QT interval includes both ventricular depolarization and repolarization times and varies inversely with heart rate. A rate related (corrected) QT interval i.e., QTc can be calculated as QT/R-R and is normally equal to 0.44 s. QT interval is dependent on duration of depolarization and repolarization of action potential. Prolonged QT interval is considered to be indicator in increased risk of ventricular arrhythmias and sudden death, as in the case with diabetic hearts (Veglio et al., 2004; Sawicki et al., 1996). In the present investigation QT interval of exercise control was reduced which correlates with decrease in the heart rate. Deca-durabolin and MEAP prolonged this QT interval as well as QTc interval except at a dose of 200 mg kg\(^{-1}\). The effect of deca-durabolin and MEAP on the heart needs further investigation especially with respect to the sodium and potassium channels. Apparently the prolongation of QT and QTc interval appeared to be without any pathological significance.

Atrial depolarization is shown by P wave in the ECG. In the present study, endurance training non-significantly reduced the P wave suggesting mild (non-significant) decrease in the atrial contraction. Treatment with deca-durabolin and MEAP (100 and 400 mg kg\(^{-1}\)) increased the P wave duration indicating stimulant effect on the atrium. MEAP (200 mg kg\(^{-1}\)) failed to show this effect. The effect of treatment on the auricles needs further investigation.

In the present investigation systolic blood pressure and diastolic blood pressure were reduced by MEAP compared to that of control. Effect of exercise training in other models did not show significant difference (Rocha et al., 2007). The present finding is similar to the observations reported by previous researcher where no change in the systolic blood pressure was observed (Fenning et al., 2003). Deca-durabolin treatment increased the systolic blood pressure and diastolic blood pressure compared to that of exercise control. On the other hand MEAP (200 mg kg\(^{-1}\)) significantly reduced systolic blood pressure and diastolic blood pressure compared to deca-durabolin and exercise control. MEAP appears to differ from deca-durabolin because it showed significant hypotensive effect in contrast to deca-durabolin. The antihypertensive effect of MEAP needs further investigation in order to understand the mechanism of action.

Endurance training resulted in decrease in max dp/dt in exercise control rats, whereas deca-durabolin increased max dp/dt. MEAP (200 mg kg\(^{-1}\)) on the other hand was not effective in increasing max dp/dt compared to exercise control and showed significant reduction compared to deca-durabolin. Left ventricular max dp/dt and min dp/dt represent the maximum and minimum rate of pressure changes in ventricle respectively. Peak dp/dt has been used as an index of ventricular performance. However it is known to be load dependent and infer to hemodynamic parameters defined by pressure volume relationship and increase in contractility is manifested as an increase ventricular max dp/dt during isovolemic contraction. However dp/dt is also influenced by preload, after load, heart rate and myocardial hypertrophy. Hence the relationship between ventricular and diastolic volume and max dp/dt is a more accurate index of contractility than max dp/dt alone. Similarly an increase in diastolic function or an increase in relaxation causes increased min dp/dt during isovolemic relaxation. Hence min dp/dt has been used as a valuable tool in the analysis of isovolemic relaxation. In the present finding reduction of max dp/dt as result of exercise appears to have been reversed significantly by deca-durabolin. The observed effect appears to be due to
increase in the heart rate observed in the deca-durabolin treated animals. The effect of MEAP appears to be significantly reduced compared to deca-durabolin. MEAP at 200 mg kg\(^{-1}\) reduced the heart rate significantly but the other two doses restored the heart rate to that of control. The results reflect the differences in the actions of deca-durabolin and MEAP with respect to max dp/dt wherein deca-durabolin showed more effectiveness compared to MEAP. Similar findings were observed in case of min dp/dt.

In conclusion, MEAP showed some distinct similarities and differences on the biochemical markers as well as hemodynamic parameters when administered orally in rats trained to undergo strenuous exercise as running on the moving belt of treadmill. The effect of MEAP on various hemodynamic parameters clearly indicated that MEAP (200 mg kg\(^{-1}\)) appears to be the optimum dose. The higher dose of MEAP (400 mg kg\(^{-1}\)) did not increase effect and the marginal benefit can be seen. The 8 weeks administration of MEAP lowered the serum cholesterol levels and HDL levels whereas, the opposite effect was seen in the serum LDL levels and triglyceride levels. Decrease in cardiac hypertrophy and serum CKMB confirms the cardioprotective effect of the drug. Significant elevation of testosterone level was observed at MEAP (200 and 400 mg kg\(^{-1}\)). The results of serum creatinine levels indicated that the optimum dose of MEAP (200 mg kg\(^{-1}\)) was equipotent to deca-durabolin in reducing serum creatinine. The electrocardiographic changes like prolongation in QT and QTc intervals were seen with the treatment though, there was no pathological significance. The hypotensive effect observed with extract treatment needs further investigation.

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