ANTIFATIGUE EFFECT OF *MURRAYA KOENIGII*

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Summary

To study the anti-fatigue effects of *Murraya koenigii* (MK) in mice weight loaded forced swimming test model (WFS) was used.In which albino mice were treated with standards Imipramine (10 mg/kg i.p) and Ashwagandha (100 mg/kg p.o) and three extracts of *Murraya koenigii* which were administered at doses of 50,100 and 200 mg/kg/day p.o. for 21 consecutive days. The levels of serum glucose, triglyceride (TG), lactate dehydrogenase (LDH), blood urea nitrogen (BUN), muscle and liver glycogen contents and swimming endurance period were determined after weight loaded forced swimming test on 21st day of treatment. Three extracts of *Murraya koenigii* at 3 different doses variably lengthened the swimming endurance period and prevented the occurrence of hypoglycemia and prevented depletion of muscle and liver glycogen content, while lowering the stress induced raised levels of serum corticosterone. Serum triglyceride ,LDH and BUN levels were also observed to be decreased significantly as compared with stress control group, Comparatively among all three extracts the most significant effect was observed with MKM (200 mg/kg) treated group. The studies revealed that *Murraya koenigii* enhanced the swimming capacity of mice by increasing fat utilization along with conservation of the glycogen stores and maintenance of the blood glucose homeostasis there by reducing the accumulation of lactic acid and ammonia thus delaying the occurrence of fatigue.

Key words : Antifatigue ,Weight loaded forced swimming test ,glycogen,*Murraya Koenigii*

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Introduction

Fatigue, the seventh most common symptom in primary health care (1) is one of the least understood conditions in medical science. Chronic fatigue is an illness characterized by profound disabling fatigue accompanied by numerous neurological and psychosomatic complaints (2,3) In this illness various neuroendocrine abnormalities contribute to the impaired energy and mood. (4).The subjective sensation of fatigue is defined as an overwhelming sense of tiredness, lack of energy or feeling of exhaustion. Fatigue is a complex symptom of physical and mental fatigue . Physical fatigue is thought to be accompanied by deterioration in performance and a mental; fatigue by depression (5,6) On exposure to a stressful stimulus fatigue is perceived as a threat to the organism's homeostasis and elicits a variety of symptoms encompassing behavioral, biochemical and neuro-chemical aspects.

Due to lack of diagnostic tests to identify etiology of fatigue ,treatment is based only on symptoms. There is no firmly established treatment recommendations for this condition. (7) and the available therapies in modern medicine are limited ,Thus the exploration of potential alternative therapies from traditional medicine is prerequisite to overcome fatigue. In traditional system of medicines MK has been claimed as tonic and is highly valued as folk medicine and functional food. MK is known to be a rich source of carbazole alkaloids (8) and is reported to possess various biological activities such as antitumor ,antioxidative, antimutagenic and antiinflammatory etc (9,10).but its anti fatigue effect is not yet investigated. Present study was designed to evaluate ani fatigue potential of various extracts of the plant *Murraya Koenigii* by using weight loaded forced swimming test model in mice .

Material and Methods

Animals

Male albino mice weighing between 20 - 30 g were housed under standard laboratory conditions and maintained on natural light and dark cycle and had free access to food and water. Animals were acclimatized to laboratory conditions before the experiment. Each animal was used only once. All the experiments were carried out between 09.00 and 15.00 h. The experimental protocols were approved by Institutional Animal Ethics Committee (IAEC).Under controlled conditions of temperature (22±1 ◦C), humidity (about 60%), and lighting (light from07:00 to 19:00 h) they were given free access to water and a commercial diet (Chakan).The care and treatment of experimental animals conformed to the guidelines for the ethical treatment of laboratory animals.

Preparation of medicinal plant extracts

Medicinal plant extracts were prepared from the air dried leaves of *Murraya Koenigii* Firstly, they were powdered and then separately extracted with Methanol (MKM) , 50% ethanol (MKHA) and water (MKAQ) using soxhlet apparatus Three extracts were concentrated under reduced pressure at 45–55 $^{\circ}$C, and then used as test samples. These test samples were dissolved or suspended in distilled water using 0.1% Sodium CMC. Oral suspensions containing 10 mg/ml of the aqueous, methanolic and hydroalcoholic extract of *Murray.Koenigii* were administered to mice.

Experimental design

After an adaptation period for a week, Albino mice (104) were randomly divided into thirteen groups, containing eight animals. in each group .Two standard groups were treated with Imipramine HCL (Sigma Aldrich) (10 mg/kg i.p.) and Ashwagandha (Ashvagandha Himalaya) (100 mg/kg ,p.o) respectively and. The mice in the nine treatment groups were orally administered with three extracts (MKM, MKHA ,MKAQ) at dose levels 50, 100 and 200 mg/kg for each extract every day for 3 weeks, while the vehicle control and stress control group received the same volume of vehicle (0.1% sodium CMC in distilled water p.o.). The mice in all groups except vehicle control groups were made to swim for 20 min three times a week to accustom them to swimming. On 21st day 1 hr after administration of extracts and Ashwagandha and 30 minutes after administration of Imipramine mice in all groups except vehicle control group were subjected to weight loaded forced swimming test .Animals in all groups were fasted overnight for blood sampling from retro orbital plexus and were sacrificed at the end of study for isolation of liver and gastrocnemius muscle.

The weight loaded forced swimming test (11)

Forced swimming capacity of mice was measured in acrylic plastic pool (65 × 45) filled with water to a depth of 40 cm (13) The temperature of the water was maintained at 34ºc ± 1ºc. The mouse was loaded with a tin wire (8 % body weight) which was attached to the tail. The swimming time to exhaustion was used as the index of the forced swimming capacity. Swimming time to exhaustion was regarded as time spent by the mouse floating in water with struggling and making necessary movements until exhausting its strength and drowning .Exhaustion was determined as a failure to swim and the mice were assessed to be exhausted when they failed to rise to the surface of water to breathe within a 7s period. (15).

Analysis of blood biochemical parameters

Mice were anaesthetized with ether. Blood was withdrawn from retro orbital plexus. Blood serum was obtained by centrifugation at 3000 rpm, 4 ˚C for 10 min. The levels of serum corticosterone, glucose, triglyceride , lactate dehydrogenase (LDH) and Blood urea nitrogen (BUN) were determined by using commercial kits (Erba diagnostics and Agappe diagnostics)

Analysis of tissue glycogen contents

Immediately after withdrawal of blood , liver and gastrocnemius muscle were quickly dissected out and were immediately homogenised in Trichloro acetic acid (TCA). The glycogen content was measured spectrophotometrically (16). Briefly, the homogenate was centrifuged. The supernatant fluid was filtered and 1 ml. of the filtrate was pipetted into a 15 ml.centrifuge tube. To each tube 5 ml of 95% ethanol was added and allowed to stand overnight at room temperature. Tubes were centrifuged at 3000 r.p.m. for 15 minutes. The clear liquid was gently decanted and packed glycogen was dissolved by addition of 2 ml. of distilled water to which 10 ml. of anthrone reagent was added while placing the tubes in ice cold water bath. After all tubes reached the temperature of the cold water, they were immersed in a boiling water bath for 15 minutes and then removed to a cold water bath and cooled to room temperature. The absorbance of the solution was determined at 620 nm by spectrophotometer (V-530, Jasco Co., Japan).

Statistical analysis

Data were analyzed using Graph pad. The results were expressed as the mean ± SEM. Statistical significance between groups was tested using one-way ANOVA followed by Dunnet's test or student's unpaired t-test as appropriate using a computer-based program.

Results

Table no1:Effect on forced swimming capacity.

Groups	**Mean SwimmingTime to exhaustion (minutes) ±SEM**
Stress control	118.88 ±8.21
Imipramine 10mg/kg	198.87* ±24.24
Ashwagandha 100mg/kg	190.37* ±19.64
MKAQ 50 mg/kg	117.12 ±14.55
MKAQ 100 mg/kg	165 ±19.93
MKAQ 200 mg/kg	151.62 ±16.61
MKHA 50 mg/kg	135.87 ±17.49
MKHA 100 mg/kg	188.87 ±21.57
MKHA200 mg/kg	192.62* ±21.57
MKM 50 mg/kg	140.87 ±19.31
MKM 100 mg/kg	191.5* ±15.39
MKM200 mg/kg	206.12** ±18.35

n=8 ,Data are expressed as mean ± SEM, **=p<0.01,*=p<0.05 when compared with stress control group (one-way ANOVA followed by Dunnett's t-test)

Table no1 shows significant difference in the swimming time to exhaustion between the stress control group and treatment groups. The swimming time to exhaustion of the standards Imipramine and Ashwagandha were found to be increased significantly (P<0.05) to an extent of 67.29% and 60.14% respectively. In groups treated with MKM (100 and 200 mg/kg) the swimming times to exhaustion (61.09% (P<0.05), 73.39% (P<0.01) , respectively) were significantly longer than that of the stress control and in groups treated with MKHA (200 mg/kg) swimming times to exhaustion were found to be increased significantly (P<0.05) to an extent of 62.04%. Where as in groups treated with MKAQ (100mg/kg) increase in swimming time to exhaustion was observed to be 38.80% as compared to stress control group but was not found to be statistically significant.

Table no 2: Effect on serum corticosterone levels.

Groups	Mean serum corticosterone levels (ng/ml) ±SEM
Vehicle control	9.62 ±1.64
Stress	24 ±3.12
Imipramine 10mg/kg	19.61 ±2.38
Ashwagandha 100mg/kg	12.32* ±2.48
MKAQ 50 mg/kg	22.67 ±5.1
MKAQ 100 mg/kg	17.12 ±3.5
MKAQ200 mg/kg	17.96 ±2.64
MKHA 50 mg/kg	20.7 ±3.02
MKHA 100 mg/kg	15.91 ±2.27
MKHA200 mg/kg	13.47* ±3.58
MKM 50 mg/kg	23.03 ±3.24
MKM 100 mg/kg	14.13* ±2.44
MKM 200 mg/kg	12.62* ±2.09

Data are expressed as mean ± SEM, **=p<0.01,*=p<0.05 when compared with stress control group, #=p<0.01,$<0.05 when compared with vehicle control group (one-way ANOVA followed by Dunnett's t-test or Student's unpaired t-test as appropriate)

Weight loaded swimming is a stressful exercise leading to physical and mental stress which was indicated by significant increase in blood corticosterone levels in stress control group as compared to vehicle control group. On treatment with MKM (100 and 200mg/kg) moderately significant (P<0.05) inhibition of stress induced increase in levels of blood corticosterone was observed to an extent of 41.09% and 47.39% respectively which were comparable with the effect produced by standard drug Ashwagandha (48.64%) Treatment with MKHA (200mg/kg) also produced moderately significant effect (43.85%) however effect observed on treatment with MKAQ was not statistically significant

Table No 3: Effect on gastrocnemius muscle and liver glycogen content in weight loaded forced swimming test

Groups	Mean Liver glycogen (mg/gm) ±SEM	Mean Muscle glycogen (mg/gm) ±SEM
Vehicle control	4.93 ±0.53	1.70 ±0.18
Stress	2.08 ±0.36	0.59 ±0.08
Imipramine	2.99 ±0.45	0.89 ±0.11
Ashwagandha 100mg/kg	3.78 ±0.41	1.43 ±0.29
MKAQ50 mg/kg	2.16 ±0.44	0.56 ±0.15
MKAQ100 mg/kg	2.99 ±0.62	0.73 ±0.11
MKAQ200 mg/kg	3.18 ±0.68	1.29 ±0.24
MKHA 50 mg/kg	2.75 ±0.51	0.69 ±0.13
MKHA 100 mg/kg	3.10 ±0.39	1.39 ±0.19
MKHA 200 mg/kg	3.70 ±0.45	1.37 ±0.22
MKM50 mg/kg	2.74 ±0.43	0.73 ±0.12
MKM100 mg/kg	3.63 ±0.28	1.37 ±0.19
MKM 200 mg/kg	4.04 ±0.50	1.53 ±0.26

Data are expressed as mean ± SEM,**=p<0.01,*=p<0.05 when compared with stress control group, #=p<0.01,$<0.05 when compared with vehicle control group (one-way ANOVA followed by Dunnett's t-test or Student's unpaired t-test as appropriate)

The contents of gastrocnemius muscle glycogen are shown in Table:3. It has been well established that during prolonged exercise the development of fatigue is closely related to the depletion of glycogen stores both in skeletal muscle and liver (18 ,19) In the present study, the content of muscle glycogen in stress control group was tended to be significantly lower (65.07%) as compared to vehicle control group. Treatment with MKM at dose 200mg/kg significantly (P<0.01) restored muscle glycogen content (156.84%) and at dose 100 mg/kg it showed moderately significant effect (p<0.05,130.2%). Treatment with MKHA at doses 100 and 200 mg/kg produced moderately significant (P<0.05) effect (133.68% and 130.52% resp.).On treatment with MKAQ moderately significant effect (P<0.05,117.89%) in restoring levels of muscle glycogen was observed only at dose of 200mg/kg.Simultaneously liver glycogen content was also observed to be declined significantly to an extent of 57.79% in stress control group. Whereas in treatment groups prevention in depletion of liver glycogen content was observed to be moderately significant (P<0.05) with the treatment of MKM at doses100 and 200mg/kg(74.17% and 94.29% resp) and MKHA at dose

200mg/kg (77.77 %) Liver glycogen content observed with MKAQ treated group was not found to be statistically significant at any one of the three doses.

Table no :4 Effect on various biochemical parameters:

Groups	Mean glucose (mg/dl)) ±SEM	Mean triglyceride (mg/dl)) ±SEM	Mean LDH (IU/Lit) ±SEM	Mean BUN (mg/dl) ±SEM
Vehicle control	85.06 ±5.54	77.71 ±3.82	4166.63 ±232.74	17.30 ±1.62
Stress	45.18 ±4.61	68.30 ±5.17	5459.88 ±148.80	26.21 ±2.23
Ashwagandha 100 mg/kg	77.36** ±6.30	66.53* ±5.12	4111.25 ±223.46	16.44** ±2.64
Imipramine 10mg/kg	69.78 ±8.64	46.31 ±5.10	4523 ±274.05	21.45 ±3.02
MKAQ 50 mg/kg	47.91 ±5.31	62.05 ±7.63	5192.63 ±216.84	24.69 ±2.83
MKAQ 100 mg/kg	59.83 ±6.01	52.30 ±5.63	5262.13 ±181.31	25.08 ±2.65
MKAQ 200 mg/kg	69.94* ±6.74	54.63 ±6.37	4602.88* ±299.56	23.26 ±2.48
MKHA 50 mg/kg	51.91 ±6.70	59.71 ±6.78	4753.88 ±332.60	22.86 ±1.18
MKHA 100 mg/kg	72.60* ±7.05	45.83* ±5.26	4424.75* ±263.87	17.42* ±1.95
MKHA 200 mg/kg	74.51* ±6.40	47.81 ±6.17	4444.88* ±318.96	16.51* ±2.26
MKM 50 mg/kg	56.29 ±6.27	68.31 ±5.16	4827.63 ±297.07	23.50 ±2.39
MKM 100 mg/kg	70.93* ±6.50	44.49* ±6.08	4484.5* ±242.19	17.06 ±2.85
MKM 200 mg/kg	78.48** ±6.53	42.79** ±6.56	4329.63** ±255.23	16.14* ±2.14

Data are expressed as mean ± SEM,**=p<0.01,*=p<0.05 when compared with stress control group, #=p<0.01,$<0.05 when compared with vehicle control group (one-way ANOVA followed by Dunnett's t-test or Student's unpaired t-test as appropriate)

Effect on blood glucose levels

The homeostasis of blood glucose plays an important role in prolonging endurance period during exercise thus it is an important biomarker which illustrate the speed and degree of fatigue development. As shown in Table no 4 , on subjecting to weight loaded forced swimming blood glucose level of stress control group was observed to be significantly lower (46.89 %) than that of vehicle control group (unstressed). Whereas the blood glucose levels of MKM (100 mg/kg and 200 mg/kg) treated mice were significantly higher than that of the stress control group ($P < 0.05$ P<0.01 respectively); 57% and 73.71% higher, respectively and

was nearly equal to the increase observed with standard Ashwagandha (71.25%).In Mice treated with MKHA(100 and 200 mg/kg) the blood GLU levels were significantly ($P<0.05$) increased to an extent of 60.7% and 64.94% respectively. and in those treated with MKAQ moderately significant ($P<0.05$) rise in blood glucose level upto-54.81% was observed at dose 200mg/kg .This indicated that the bloodglucose-regulating ability of the treatment groups was improved as compared to that of in the stress control group.

Effect on Triglyceride levels

Notably triglyceride concentrations in the blood of stress control mice was higher than treated mice. (Table 4) TG levels were lowered in MKM (100mg/kg and 200mg/kg) treated mice to an extent of 34.86 % ($P<0.05$) and 37.35% ($P<0.01$) and with MKHA(100 mg/kg) to an extent of 32.9% as compared with the stress control mice. These results suggest that MK intake increased TG consumption.

Effect on LDH levels

With intense exercise, O_2 and pyruvic acid are reduced by Lactate dehydrogenase (LDH) to lactic acid (LA),which causes decrease in the contractile strength of the muscle and thus eventually induces fatigue (20). As shown in Table no 4 , the LDH levels of stress control group were observed to be increased significantly to an extent of 31.03% as compared to vehicle control group but in MKM (100 and 200 mg/kg) treated mice LDH levels were observed to be significantly ($P<0.05$ and $P<0.01$ resp.) lower than that of the stress control group , 17.80% and 20.7% lowering, respectively. Moderately significant ($P<0.05$) lowering of 18.95 % and18.59 % .in LDH levels was observed on treatment with MKHA (100 and 200 mg/kg) respectively and .15.69% decrease was found on treatment with MKAQ 200 mg/kg which is another confirmation that *Murraya koenigii* possess significant anti-fatigue effect.

Effect on BUN levels

The content of serum BUN raises with increasing exercise load. Our results in table no 4. showed the trend that BUN value of the stress control group were increased significantly to an extent of 51.5% as compared to vehicle control group. MKM (100 and 200 mg/kg) treated group showed moderately significant ($P<0.05$) lowering of BUN value (34.9%and 38.43% resp.) MKHA (100 and 200 mg/kg) treated group also showed moderately significant lowering of BUN value (20.05% and 24.% resp.) where as treatment with MKAQ failed to elicit significant lowering of BUN levels in treatment groups. The reduced protein metabolism on the treatment with *Murraya koenigii* extracts is indicative of enhanced endurance.

Discussion

The weight loaded forced-swimming test is commonly used as anti-fatigue and endurance tests (21–23) In order to clarify mechanism, blood biochemical parameters were measured in the weight loaded forced swimming test. The swimming exercise is known to induce biochemical changes in blood .(24) The weight loaded forced swimming is a strainous exercise leading to physical stress triggering the activation of pituatory adrenocortical activity and consequently, the production and secretion of corticosterone which was found to be elevated in stress control group as compared to vehicle control group , not subjected to weight loaded forced swimming (25-28) where as on treatment with MK extracts stress induced rise in levels of

serum corticosterone were found to be prevented This resulted in delaying the onset of getting stressed and thus becoming fatigued hence enhancing the ability of mice to continue the exercise for a longer period as compared to stress control group.

This rise in levels of corticosterone in stress control group might have induced prompt increase in the level of glucose in the blood . But in weight loaded swimming exercise , to sustain in water mouse is forced to continue swimming exercise against the downward pulling force of load applied to its tail for which muscles in hind limbs need energy. As a result, the available glucose in blood might have got used up rapidly by the muscle tissues as a source of energy leading to significant fall in blood glucose level in stress control group However, it is possible that a decrease in blood glucose concentration (hypoglycaemia) might have contributed in part to the occurrence of fatigue in the stress control groups . Since blood glucose is a major source of energy to both muscle tissue and the CNS specially the only preferential energy substrate to CNS for its activity which might be compromised in a state of hypo glycemia (29) hence suppression of the active functioning of the brain during exercise often leads to the inability to continue exercise. (30). Thus, the amount of blood glucose can illustrate the speed and degree of fatigue development. (31).One can observe from the Table 4 that in stress control group decreased swimming duration and rapid induction of fatigue may be result of significant fall in blood glucose levels during weight loaded forced swimming where as prolonged swimming endurance period in treatment groups at various dose levels of three extracts with relative increase in plasma glucose levels indicated the blood-glucose-regulating ability of MK in treatment groups. by maintaining availability of energy substrate i.e. glucose in blood for a longer period. This homeostasis of blood glucose plays an important role in prolonging swimming endurance period during exercise (32)

Energy for exercise is derived initially from the breakdown of glycogen in muscle and, later, from circulating glucose released by the liver as a result of glycogenolysis (33)` Thus the amount of glycogen, which reflects the source of the energy, would be more suitable marker of physical fatigue. In general, glycogen is consumed in both muscle and liver during physical fatigue. The role of hepatic glycogen is to complement the consumption of blood glucose and maintain the blood glucose in the physiologic range. Fatigue will happen when the liver glycogen is mostly consumed (34) as a result of the depletion of hepatic glycogen stores plasma glucose concentration decreases during prolonged exercise,. (35) and the ensuing hypoglycaemia could result in impaired CNS functionality Thus, the maintenance of glycemia during physical activity by hepatic gluconeogenesis and glycogenolysis is of extreme importance for exercise continuation (36) As shown in Table (3), the liver glycogen levels of treatment mice were both significantly higher than that of the stress control group ($P < 0.05$), These results indicate that exercise induced depletion of glycogen stores in liver tissues was declined in groups treated with MK extracts suggesting that the anti-fatigue activity of MK may be related to the improvement in the metabolic control of exercise and the activation of energy metabolism (37)Similarly muscle glycogen levels also demonstrate the important factor in determining endurance during exercise and depletion of muscle glycogen plays a key role in development of fatigue and exhaustion. Depletion of these deep carbohydrate stores should certainly prove limiting for any type of physical activity. Our findings indicated that the rate of glycogen depletion was decreased significantly in the MKM (100 and 200 mg/kg) ,MKHA (100 and 200mg/kg) and MKAQ (200 mg/kg) treated mice. Thus treatment with MK extracts could decrease carbohydrate utilization during exercise thus sparing muscle glycogen stores for a longer period .The possible reason may be that pre treatment with *Murraya koenigii* might have increased the content of liver and muscle glycogen in mice

before subjecting to exercise by improving glycogen reserves, or by reducing the consumption of glycogen during exercise, or might be both. thus resulting enhanced swimming capacity and delay in the onset of fatigue in mice.

On the other hand, TG levels in the blood were found to be declined in MK treated mice which indicated that while the glycogen stores were conserved in both muscle and liver by MK intake, the good performance during the swimming test was due to the energy supplied by TG degradation in the treated mice. In view of the lowered plasma level of triglyceride in the MK extracts treated groups as compared to the stress control group, it would seem reasonable to suggest that lipid utilization may be increased in treatment group as an alternate source of energy. (38-39) .

The present results suggest that enhancement of exercise capacity in treatment with MK extracts could be due to increase fat utilization of mice during swimming. , allowing glycogen sparing and therefore delay in the onset of fatigue . (40,41)

The response to exercise in mammals begins with an increase in aerobic muscular activity, which switches over to anaerobic metabolism if the exercise is intense in which O_2 and pyruvic acid are reduced by lactate dehydrogenase (LDH) to Lactic acid (LA). (42) and that of increase in lactic acid presents a fatigued condition Accumulation of LA decreases the pH, affecting the skeletal muscle system function. By decreasing the contractile strength of the muscle that eventually induces fatigue so the levels of LDH in blood were measured. As shown in Table (4), the LDH levels of stress control group were observed to be increased significantly as compared to vehicle control group. In MKM MKHA and MKAQ treated mice serum LDH levels were found to be significantly lowered than that of the stress control group. The results suggest that MK can reduce the production of blood lactic acid during exercise. which is another confirmation that MK has an anti-fatigue effect.

Urea is formed in the liver as the end product of protein metabolism. During digestion, protein is broken down into small peptides and amino acids. The amino acid nitrogen is removed as NH_4, while the rest of the molecule is used to produce energy or other substances needed by the cell (43) Circulating ammonia is taken up by the liver and most of it is detoxified in this tissue through the urea cycle;(44-45) Thus Blood urea nitrogen (BUN) , which is a product of energy metabolism, is another sensitive index of fatigue status. As shown in Table (4), the BUN levels of the treatment mice were significantly lowered by MKM and MKHA as compared to the stress control group while the decrease in MKAQ treated group was not statistically significant . The reduced protein metabolism in the MK treated group is indication of enhanced endurance

The data mentioned above revealed that the content of liver and muscle glycogen of mice in MK treated groups were higher than that of stress control group after swimming. However its detailed mechanism is not clear. The possible reason is that MK may increase the content of liver and muscle glycogen of mice post exercise by improving glycogen reserves, or by reducing the consumption of glycogen during exercise, or both. The later effect has a greater probability as triglyceride levels of treatment groups were found to be lowered thus indicating shift in metabolism from glycogen to triglycerides thus conserving energy stores such as liver and muscle glycogen. Serum urea nitrogen and LDH levels are important blood biochemical parameters related to fatigue. In our experiment, the blood LDH levels in MK treated groups were lower than that of stress control group after weight loaded forced swimming. The content of serum BUN raises with the exercise load Our results showed that serum BUN of the MK treated groups were lower than stress

control group.In conclusion, treatment with MK extracts induced anti-fatigue effects on mice and these effects were variably significant in all three extracts but the strongest effect on most of biomarkers was seen with MKM (200 mg/kg) ,The detailed mechanism of anti-fatigue properties of MK might be mediated through regulating central nervous system, antioxidant mechanism and /or improving substance metabolism and aerobic capacity. However further investigational studies need to be done to clarify the detailed mechanisms involved in the anti-fatigue properties of MK.

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