Section A-Research paper

FABRICATION OF MULTIPURPOSE EXPERIMENTAL MODEL FOR VARIOUS PHARMACOLOGICAL SCREENINGS

Krutika Sawarkar*[1], Sachin More[2], Abhijeet A.Chalkhor[3], Tejas N.Kharkate[4], Sarvesh R. Rambhad[5]

1,2 Assistant Professor, Department of Pharmacology, Dadasaheb Balpande College of Pharmacy, Besa, Nagpur, India.

3,4,5 Student, Dadasaheb Balpande College of Pharmacy, Besa Nagpur, India.

***Corresponding author's E-mail:** krutikasawarkar786@gmial.com

Abstract

Current study focus on fabrication of a multipurpose model that was used for various pharmacological screenings like: spinal cord injury, exercise in diabetes mellitus, grip strength on rats. Rotarod performance test is based on rotating rod with force motor activity being applied usually by a rat. Functions of test include evaluating balance, grip strength motor co-ordination of the rats especially in testing the effect of experimental drug. [1] The test may be useful as a sensitive indicator of trauma induced by brain injury to laboratory rodents[2]. The rota rod consists rotating cylinder, which itself is motor driven [3]. The speed of the rotating cylinder can be controlled. The newly fabricated was operate in such a way that it will utilise the rotating cylinder of rotarod as one of the roller of treadmill which will help in running the belt at desired speed. This modification can be used in the study of STZ induced diabetic rats that was forced to exercise and the effect of this exercise was monitored. There are already many studies conducted for the effect of STZ induced diabetes Mellitus where it is proven that the treadmill exercise shows significant reduction in the symptoms of said disease. [4]

Another modification on rotarod apparatus was the use of device that vertically supports the rats, so that the lower limbs of the rats are on the treadmill which was operated on the desired speed to study the effect of spinal cord injury, where the rats is forced to walk after spinal cord injury considering the fact that motor co-ordination is as similar as rotarod apparatus. [5]

The third modification was the use of the same apparatus by changing the dimensions in such a way that the ramp gets inclined at 40^o so that the rats was forced to use their grip strength while climbing the rotating ramp similar to the model available to study grip strength.

The desired inclined angle to study the grip strength was calculated by the installation of a compass that indicates all the possible angles at which the model can be inclined. [6]

Keywords: Treadmill, Spinal cord injury, Grip strength, Multipurpose pharmacological screening

Introduction

The treadmill is a popular exercise system which is used to force-train the rats. The apparatus has a simple construction such that the experimenter can vary speeds and inclinations of the runway belt. Most treadmill systems utilize shock grids to motivate the rats to keep running. However, this can result in pain-stress affecting exercise training. Some of the other methods of motivation like a gentle tap or air-puffs can be used as a relatively less stressful alternative. The treadmill consists of a motorized runway and systems to control the speed and inclinations of the treadmill.[10.] A treadmill design for small laboratory rats was described by Andrews (1965) [14] which allowed the experimenter to change the speed of the runway between 13 to 70 ft/min and inclination between 0 to 16°. Andrews' treadmill was made of a horizontal plane that could be divided into different individual lanes. The individual lanes provided the opportunity to train multiple rats at fixed training parameters such as speed and inclination. The treadmill is a simple tool which is used to evaluate the effects of exercise and different intensity training on not just physical health but also cognitive (Hwang et al., 2016)and mental health (Costa et al., 2012)[11]. Experimenters will have chance to observe the simultaneous performances of different treatment groups when using a multilane treadmill. The use of a treadmill is not limited to just physical activity (Dougherty, Springer, & Gershengorn. 2016) [12] that can often be a symptom of diseases and disorders. Further, assessment of motor and locomotion function post-recovery from injuries can also be done using the treadmill (English, Wilhelm, &Sabatier, 2011) [13]. The treadmill consists of a motorized runway cons associated systems to control the speed and inclinations of the runway. It also comes equipped with shock grids at the start of the runway that can be turned on or off as per the requirement.
Eventually, a treadmill design for small laboratory rats was described by Andrews (1965) that allowed the experimenter to change the speed of the runway between 13 to 70 ft/min and inclination between 0 to 16°. Andrews' treadmill made use of a horizontal plane that divides into individual lanes. Each individual lane provides the opportunity to train multiple rats at fixed training parameters such as speed and inclination.

Rotarod: -The rotarod, also known as the rotarod test, is used as a basic assessment tool for coordination and balance in rodents and provides one measure of locomotor ability (30, 31).
It is a pharmacological instrument/apparatus which is generally used for the muscle strength. It consists of rotating cylinder typically 3-3.5 cm in diameter for the rats. The cylinder is usually made up of solid material such as rubber. The rotation of the rod was manual design to accommodate simultaneously testing of multiple rats. The cylinder is mounted above a platform, typically at a height of 15 cm for a rats and 30cm for the rats. The cylinder is elevated above a padded landing area to reduce, the risk of injury to animals that fall.
Animals with deficits affect balance or coordination falls from the apparatus more quickly than animals with normal motor function. The rotarod has been proposed as one of a number of behavioural tests for phenotype genetically modified mice [32, 33, and 34.] The platform may be equipped with sensors that allow the device to cease rotation and recorded ending time of test when a rat contacts the platform or breaks an infrared beam. Devices was also be equipped with timer that allow for automatic recording of a time from start of the test to when the rats

contact the platform. This period of time is latency to fall, is the dependent variable of the test.

Rotarod may be classified into two basic categories, constant speed and accelerating rotarod. The cylinder of constant speed devices rotated at a set speed for example, five rotations per minute or higher. Rats are then accessed for their ability to remain on the rotating cylinder for the minimum amount of time, i.e. 1-5 minutes. Rotarod gradually increase speed over a predetermined period of time. The test can be in with a stationary cylinder or rats may be placed on a slowly moving cylinder and the speed of cylinder increased. For example, over a 1–5-minute period, the cylinder may accelerate to a maximum speed of 40rpm. [7] The cylinder is typically made of a solid substrate such as rubber. The cylinder may be modified by using stainless steel bars or wire mesh as the walking surface. With such modifications, rats were able to cling to, and rotate with, the rod. When this occurs, after one or two complete rotations, the test should be considered complete, and the time recorded. Inbred strains of rats may perform differently on the test. Rats that perform poorly on introduction to the rotarod, it may be beneficial to acclimatize the rats to the apparatus prior to beginning the study. Rats with experimentally induced lesions to the CNS and rats receiving therapeutic and experimental compounds may also be tested for motor deficits using the rotarod. Lesion rats should be allowed to recover from surgery for a minimum of 24 hrs prior to testing. Drugs and experimental compounds were administered just prior to testing.

First modification was for the purpose of study of effect of exercise on STZ induced diabetes in which the rats was forced to run on a treadmill.Type 2 diabetes, which is characterized by hyperglycemia associated with insulin resistance, is the most common metabolic disease in nearly all countries and is increasing explosively in developing countries [16].Although there are easy-to-apply methods used to define exercise intensity, such as the maximum oxygen consumption (VO2 max) and the lactate test a protocol that establishes the levels of exercise intensity (very light, mild, moderate, intense, very intense, and maximum) that meet the physiological conditions of these models is not defined in the literature. [41] There are conflicting reports regarding the effects of physical exercise on ROS generation and antioxidant levels in the hippocampus of animals with STZ-induced type 1 diabetes [42]. High-dose of STZ. The dose for a single high dose in mice ranges from 100 to 200 mg/kg-1.depending on the mouse strain, and in rats 35–65 mg/kg-1. This leads to a rapid ablation of the beta cells and hyperglycemias. However, that it has been suggested that regeneration of the pancreatic islets can occur after STZ treatment; and thus sufficient controls should be in place to determine that any improvement in glycaemia is not due to spontaneous regeneration of endogenous beta cells High-dose STZ is often used in transplantation models, where islets or putative stem cells are transplanted under the kidney capsule. It should be noted that STZ has recently been shown to cause lymphopenia and a relative increase in T-regulatory cells, which could interfere with the interpretation of studies involving immune tolerance to transplants. Streptozotocin (STZ) can be administered as multiple low doses over 5 days to induce insulitis in mice or rats. Doses range from 20 to 40 mg·kg-1 per day, depending on the species and strain. A reduction in islet number and volume is apparent with concomitant reduction in insulin secretion capacity. [43]

The metabolic disorder of diabetes leads to characteristic complications contributing to the development of micro- and macrovascular atherosclerosis [17].Patients with diabetes not only are predisposed to stroke but also often suffer exacerbated post stroke damages [(18, 19, 20)].In accordance with these clinical observations, basic studies have also demonstrated that the diabetic state aggravates cerebral ischemic injury in both type 1 [(21, 22, 23)] and type 2 diabetic animal models [(24, 25).] The major causes exacerbating post ischemic cerebral damage with diabetes are considered to be elevated levels of oxidative stress and inflammatory cytokines. Sustained hyperglycemia has been suggested to produce excessive intracellular reactive oxygen species (ROS) and enhance systemic oxidative stress, which accelerates apoptotic and proinflammatory processes in the brain tissue [26]. Furthermore, visceral obesity associated with type 2 diabetes also has been reported to induce production of inflammatory cytokines and augment oxidative stress via dysregulation of adipose tissue function [(27, 28).] Impairments in motor coordination and balance in the mice were assessed by the accelerating rotarod test [36]. Mice were placed on a 3 cm diameter cylinder of the rotarod apparatus (MK-630B; Muromachi Kikai Co. Ltd., Tokyo, Japan) and accelerated from 4 to 40 rpm in 5 min. Trials began by placing the rats on the rod and starting the rotation. Each trial ended when the rats fell off the rod and the latency was recorded. Mice were given five trials 2 days before H/I. Then the rotarod performance was tested for three trials 2 h before and 24 h after H/I. [29] Further, the rotarod is modified into a treadmill. As mentioned in the above paragraph the rotarod possess a rotating cylinder, which is powered by a motor. The same motor can be used as one of the roller in treadmill. The belt was covered on the rollers as same as that in the gym treadmills for humans. The belt was move and rotates when the rollers are powered. The rats previously induced with STZ injection was placed on to the treadmill belts, and the treadmill was turned on. The speed of the treadmill was kept optimum so that the rats was try to move in opposite direction to that of the rotating treadmill belt. The rats are forced to run on the belt, for a specific time period. At the beginning the speed and the time duration for exercise is kept low or less and progressively increased, so that the rats gets well adapted to the exercise. The purpose of this exercise is to have evidence of effect of exercise when rats is suffering for a state of type -2 diabetes.

Second modification was the use of above modification of treadmill and bridging stainless steel rods on the treadmill. This new modification was used in the study of spinal cord injury in rats. The strings are suspended and to the string’s harnesses are attached. The harnesses were such that it supports the rats of which the spinal cord is injured previously. Only the lower limbs of the rats were on the treadmill belt. The treadmill belt was then powered and speed was adjusted according to the protocol. When the treadmill is turned “ON” the treadmill was come into motion and the rats is hung in such a way that the lower limbs was in the contact of the treadmill belt. The rats were hanged on a suspended harness back-pack. This modification was operated in such a way that the rats was forced to use his lower limbs with the moving treadmill belt.The ability of the lumbosacral spinal cord to generate locomotor movements of the hindlimbs after a low thoracic spinal section has been shown in many species. In contrast, rats are not able to re-express sustained locomotion with their hindlimbs after a spinal lesion unless drugs or other treatments are used. However, rats spinalized as neonates can walk with their hindlimbs on a treadmill. A detailed description of the kinematics of locomotion in adult normal and spinal rats is still lacking (Clarke and Still). In an open-field situation, in which rats move around with their forelimbs, it is important to know to what extent the isolated lumbar cord can produce locomotor movements when nonspecific stimulation of the abdomen or the tail is provided as a consequence of these forelimb movements. Thus, we propose here to determine first, the main characteristics of murine locomotion on a treadmill, and second, to what extent rats can express a locomotor pattern after a complete spinal transaction. Some of the results have been reported previously in abstract form. [35]

Spinal cord injury (SCI), a medical problem, is associated with severe disability and high rates of deaths. As there is no definite treatment for SCI, various research studies are ongoing including experimental models to understand the anatomical and biological events involved in SCI and repair, also to test the safety and efficacy of potential therapies. Animal models were investigated by in vivo methods, used to analyze events under controlled conditions. To select an appropriate model and test a specific hypothesis, all existing SCI rats' models and related outcome assessments were considered. [36]

Spinal cord injury (SCI) is recognized as one of the main causes of disability. Along with the direct SCI consequences associated with a loss of motor, sensory, and autonomic functions, there is a risk of secondary processes that may aggravate injury and lead to muscle atrophy, chronic pain, urinary tract infection, and pressure ulcers. Spinal cord injuries in rats have become the main model used to evaluate the strategy of experimental treatment of SCI. [37, 38]

Third modification was use of same modification but without the bridging of stainless-steel rods. The treadmill was uplifted from one side in such a way that it gives inclined angle between 40-45^{o}. After setting the desired angle the treadmill was turned on which the rats was placed and forced to run along with the treadmill conveyor belt with gradually increasing speed. In this modification the rats was forced to use his whole-body strength (mainly his grip strength). This test usually demonstrates the muscle strength of both forelimbs and hindlimbs.Muscle diseases usually lead to alterations in skeletal muscle function. As primary phenotypic screening, non-invasive in vivo test are hence considered important for evaluation of muscle performance. Further to perform the treadmill exhaustion test, this evaluates exercise capacity and endurance, and the limb grip strength assay, which measures muscular strength. The limb grip strength assay was used. A horizontal grip–that is grasped by the mouse–to measure the maximum force that is required to make the mouse release it. These tests were customized to evaluate muscle performance under different situations, such as after therapeutic interventions or regeneration. Exercise capacity was commonly evaluated by comparison of both speed and distance values between the rats of interest and their controls. [39]

A grip strength test was used to evaluate both forelimb and hindlimb functions. Grip strength measurement is important because it can predict any motor deficit in the limbs after a SCI. Anderson and colleagues showed that after a complete unilateral hemisection, rats lose the gripping ability permanently. Acclimatization of rats to the testing apparatus is necessary before starting the test. In grip strength test, rats are forcibly motivated to perform the task. [40]

Fig.1 Side View

Fig. 2. Front View

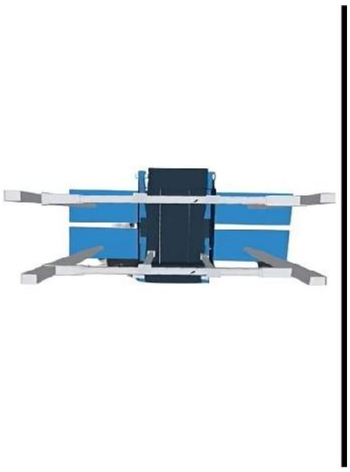

Fig. 3. Top View

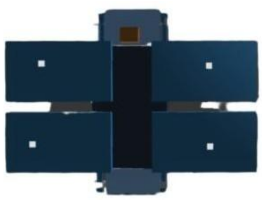

Fig. 4. Bottom view

Percentage Calculation of Blood Glucose Level Changes

% decreasing= First (Blood Glucose level)-Final (Blood Glucose level)/First Glucose level x 100%

Diabetes in rodent as a fasting glucose level >250mg/dL

Table No.1

Rats/ranges	Fasting	Post meal
Rat	71-111 mg/dl	102- 142 mg/dl
mice	80-100 mg/dl	98-120 mg/dl

CONSTRUCTION: The rotarod apparatus was converted into a multipurpose model. This model was comprised of existing motor of rotarod. This motor was placed at one end of the multipurpose model. This motor was providing energy to one of the rollers of the treadmill. The treadmill belt was covered on the two rollers. One roller was powered with the rotarod motor while the other one was free. The runway is made up of textured belt that facilitates rats grip. The individual lane havea dimension of 45x6 x12.7cm and are separated by non-reflective black barrier.The Treadmill Harness is composed of a harness and body weight support mechanism. The rats are fastened into the harness, which consists of a Velcro strap vest having two holes for the limbs. The harness is then attached to the bodyweight support mechanism at both rostral and caudal ends. The weight supported during the Treadmill training is determined by adjusting the weight support springs. The body weight support mechanism fixes the rats to a position on the treadmill while training. The Treadmill Harness is composed of two parts: harness and body weight support mechanism. The harness is made of a fabric designed as a vest with Velcro straps to fit the rats' snugly. Additional straps are attached to the end of the vest that creates a hook-and-loop mechanism to lift the hind limbs of the rats when attached to the weight support mechanism.

Motor - The speed of the lane can be varied between 0-80 meters per minute with acceleration increment of 0.1 meter per minute. The runway incline can be varied between 0-90^0 . Belt texture is such that it facilitates the rats grip, the noise should be less than 40 db and puff accessory for aversive stimuli.

Data analysis: -The treadmill apparatus is used for forced exercise training in rats. It can also be used to compare performance differences between rats of different strains, sexes and ages. The following observations can be interpreted such as-

- Running time
- Distance covered
- Maximum speed achieved
- Number of apathetic stimulations
- Average position on the runway
- Time to exhaustion/fatigue [9]

Fabrication

Materials

Treadmill base and walking belt- We purchased a second-hand human treadmill. The motor, overall base, and control panel was dismantled. We used the belt from a motorized gym treadmill.

Rats-specific frame with lanes: To make frame and running lanes, we used carpenter's pine wood coated with a plastic paint to allow for sterilization/cleaning using wipes. We made the lane dividers using white acrylic panels with dimensions (45 cm x 90 cm x 0.2 cm). The overall frame was covered by a clear acrylic panel (60 cm x 91 cm x 0.2 cm).

Firstly the treadmill was dismantled after dismantling the treadmill; we shortened the walking board and main frame so that it fit the desired sized belt. The extent that needed to be cut from the rear of the equipment was calculated as the differences between the long, adult sized belt and the child belt, divided by two. To make the treadmill lightweighed based on the calculated sizes using circular and metal hand saws. To decrease the treadmill's weight and increase its portability, we decided to remove as much as material possible to reduce the overall weight, which involved shortening the handrails. The wiring was removed first. Then after by disconnecting the control panel from the tread mill's circuit board (located in the motor cover) before the wiring was fished out. Handrails were cut using the metal hand saw to the height of the motor cover. The control panel was reconnected by pulling the wiring back through the shortened hand rail. Keep the excess wire, providing an extension to increase mobility when using the control panel.

The treadmill was reassembled by using the shortened walking board and smaller treadmill belt. Once the belt was in place, adjustments were made to keep it centred and prevent shifting to either side. To achieve this, we used an Allen key on the rear adjustment bolts located in the left and right rear caps. If the belt shifts to the left, the left adjustment bolt becomes tight by half a turn while the right adjustment bolt was loosened by half a turn. Once the belt's large shifts had been minimized, the adjustments were fine-tuned until the belt remained centred. If the belt shifts to the right, this procedure would simply need to be reversed.

Preparing frame with lanes: With a shortened treadmill ready, a frame was created with running lanes tohold the rodents during trials. When designing the lanes with in the frame, it is important that they meet the correct specifications for the target rats; several frames of different sizes can be prepared to accommodate various species. The maximum number of lanes depends on the width of the treadmill belt and the size of the rats. The design should allow for as many s as possible to run during each trial. For rats, recommended lane dimensions are 41 cm long by 5.16 cm wide by 6 cm tall; for rats, 61 cm by 10.14 cm by 14 cm. The frame for the lanes shall be slightly wider than the treadmill belt but should not exceed the width of the rear caps the brackets were used to assemble the frame, though the method of assembly can vary as long as the dimensions of the frame are met. Insert the lane dividers, cut small slits (2 mm wide by 4 mm deep) for each panel across the height of the frame. The lane dividers were measured, marked and cut from the white acrylic panel. The depth of both slits (6 mm total) shall be added to the length of the dividers. A variety of methods can be used to cut the acrylic panel-we used a ceramic tile saw.
Bottom- To place the frame on its base, we first screwed two “L” shaped brackets to the bottom left and right sides of the frame, 5 cm from the front and 15 cm from the back. Then, frame was placed on the treadmill and centred it with the belt. Using a pencil, we did mark of

holes on the walking board according to the four "L" brackets. Using a drill bit, we drilled halfway through the top side of the walking board and placed four long bolts through these holes from the bottom out the top; the bolts were kept in place by a lock washer and a nut. The required height was kept greater than the diameter of the rats' tail, as this will prevent the rodent from getting stuck between the frame and the walking belt. Place the frame on the treadmill's bolts; we used a third set of nuts to secure the frame in place. To finalize the exhaustion area in the frame, with traced the line at the same height as the walking board onto the back of the frame. After that the frame was removed and installed finishing nails at the marked height at a one-centimetre distance with the exception of immediately below the lane dividers, where the finishing nails should be higher. This arrangement makes the back of the treadmill safe and uncomfortable to the rats. In addition, wire brushes fit between the nails to condition the rats to use the treadmill. Finally, we drilled a hole through the centre of each lane through which a small air pump can be used to blast air at the rats and thus further motivate them to keep running.

Conclusion: In this study paper we have discussed how there are various instrumental models available for the pharmacological screenings of diabetes induced in rats, spinal cord injury in rats and grip strength in rats and how these all can be integrated into a single model which can be used for the screenings of all these activities for rats. The purpose behind constructing such integrated model was to reduce need for various different models and to make it cost effective.

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