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Preclinical Research Tool Innovation in Resource-scarce Setting – A Case Study of a Mouse Anxiety Multi-test Appararus

Umarudeen Ajibola Monisola [a*] and Khan Ferhat [b]

[a] Department of Pharmacology and Therapeutics, Faculty of Basic Clinical Sciences, College of Health Sciences, University of Abuja, Federal Capital Territory, Nigeria.
[b] Department of Pharmacology and Therapeutics, Faculty of Pharmaceutical Sciences, Ahmadu Bello University, Zaria, Nigeria.

Authors' contributions

This work was carried out in collaboration between both authors. Both authors read and approved the final manuscript.

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ABSTRACT

One of the factors responsible for the high prevalence and attendant socio-economic burden of anxiety disorders is the paucity and slow pace of discovery of new anxiolytic drugs to complement or replace the existing ones due to high attrition rates and poor translation of preclinical anxiolytic drug discovery efforts to clinical usage. This scenario is viewed to arise from certain factors including the inherent anxiety sensitivity idiosyncrasies of most used individual classical anxiety tests/models when used as single assays. This thrust of this study is to invent a mouse multi-test that will be devoid of this limitation. Previous attempts to overcome this limitation by testing and retesting experimental mice on multiple individual paradigms on different times were soon encumbered by one-trial tolerance phenomenon on subsequent trials. Although a multi-test

**Corresponding author: E-mail: ajibola.umarudeen@uniabuja.edu.ng;*

apparatus invented by Ramos comprising a light-dark, plus-, and open-field mazes is largely devoid of the limitations observed with single anxiety apparatuses, the central area of the elevated plus maze in the middle of the triple test still retains some ambiguity and errors in the behavioural thus generated. A novel mouse anxiety multi-test device comprising light-dark maze, zero-maze, and marble-burying mazes, dubbed U. of A. Mouse Anxiety Multi-test, that would be devoid of the limitations of the existing mouse single and multi- anxiety tests on one hand, and would potentially exhibit greater and richer sensitivity to anxiety-related behaviours in the mouse, was conceptualised. The fabrication of this composite anxiety test tool was made from locally sourced materials using simple tools requiring minimal space. The new mouse anxiety triple so generated is cost-effective – costing about 4 percent of the worth of its foreign counterparts. It is environmentally safe, portable, and operationally simple. Initial behavioural assessment of the tool indicate it can generate state anxiety in mice. This innovation is a potential asset to preclinical research in anxiolytic drug discovery. However, there is the need for its further pharmacological evaluation and automation.

Keywords: Anxiety; mouse; triple test; U. of A.; appropriate technology.

1. INTRODUCTION

The prevalence of anxiety disorders – with their attendant socio-economic burden – is not only high but on the increase, globally. In contrast, the pace of discovery of new anti-anxiety agents to replace and/or complement the existing ones in mitigating the high prevalence and the negative health impact of these disorders has been slow due to, among other factors, high attrition rate and poor translation of preclinical anxiolytic drug discovery findings to clinical use [1,2]. This scenario has restricted anti-anxiety treatment choices to a few chemical classes – majorly the gamma amino butyric acid (GABA) – and serotonin-linked anxiolytics – thus, widening the therapeutic gap in the management of these disorders [2]. This calls for renewed efforts in the search for new agents that could augment or improve on the efficacy and toxicity liabilities of the existing anxiolytic drugs [2].

The weak translational proficiency of preclinical anxiolytic drug discovery research in generating clinically relevant drugs is viewed to derive from a plethora of factors including over-reliance on disease classifications based mainly on clinical symptomology as opposed to those based on combined observable behaviours and underlying neurobiological mechanisms, wrong experimental subject selection, absent or weak preclinical-clinical bidirectional research communications, poor experimental design and methodology, and inherent anxiety-behaviour detecting limitations of individual animal anxiety tests/models when used as single tools in behavioural trials [2,3,4,5]. The thrust of this study is to address the last factor by crafting a triple mouse anxiety test comprising the light dark, elevated zero, and marble-burying mazes – three of the so-called classical animal anxiety tests - with a view to minimizing/overcoming the highlighted and other limitations of the stand-alone tests.

Previously, various workers have attempted overcoming the fallouts from the idiosyncratic anxiety sensitivity failings of individual single tests/assays by recommending use of test batteries which is encumbered by the need to re-test essentially on different days, the risk of incurring temporal biases, and the development of the one-trial tolerance (OTT) [6,7,8,9,10]. To minimize the number of experimental mice needed for trials and the temporal bias that may arise from re-tests of experimental animals on different days Umarudeen et al. (2020) had proposed a quasi-simultaneous serial testing protocol whereby the animals were manually transferred from the first to the last test apparatus after a 5-minute interaction of each mouse with each of the apparatuses. Although the objective of minimizing experimental mice needed may have been achieved, this protocol is challenged on the negative impact repeated inter-test animal handling may have on the reproducibility of the data so generated [11]. To overcome the inherent shortcomings in individual single and battery anxiety tests Ramos. (2008) [12] physically combined elevated light dark (LDM), plus- (EPM), and open-field (OFM) mazes. Again, although this experimental protocol has been reported to afford the use of minimal number of experimental subjects and to avoid the development of OTT following repeated animal testing, the neutral central square of the EPM in the middle of the set-up still poses a

challenge to data harvesting and apportioning [12,13,14,15].

The proposed mouse anxiety triple test is designed to have light dark (LDM), zero- (EZM), and marble-burying (MBM) mazes physically joined together with connections between them in that order. The inclusion of EZM instead of EPM and MBM instead of OFM in the novel experimental set-up is expected to potentially showcase certain positive features in addition to the advantages already highlighted for the triple test by Ramos. (2008). Firstly, the substitution of EPM by EZM in the new proposal is expected to effectively eliminate the EPM central square-linked ambiguity in data generation and analysis [13]. Secondly, studies have shown the EZM to be resistant to the development of OTT – a major downside of EPM – on repeated trials [14,15,16]. Thirdly, the substitution of open-field test (OFT) by marble-burying (MBT) is expected to potentially increase the versatility of the novel mouse anxiety triple test on the ground that the MBT does not only exhibit predictive sensitivity to both benzodiazepine and serotonergic drugs - the two main classes of anxiolytics but also to anxiety and obsessive compulsive behaviours in experimental animals [17,18,19].

The overall goal of this study is to generate units of mouse anxiety multi-test tool that will engender detection of broad repertoires of experimental anxiety-related behaviours that are likely to enhance the validity, and hence, the translationality of preclinical anxiolytic drug research.

A huge attraction to the triple test tool being proposed is the economic advantage gained from the local fabrication of the component test tools. All fabrications were made from locally available materials thus, saving the scarce foreign exchange that would have been required to import similar test apparatuses from international markets.

2. MATERIALS AND METHODS

2.1 Materials

The following items were purchased from various markets in Gwagwalada, Gwagwalada Area Council, Abuja, Federal Capital Territory, Nigeria. Four and 2 full (4x2 m) sheets of 3-mm and 2-mm thick China-made plastic glass at N45, 000: 00k and N37, 500: 00k each, respectively (N255, 000: 00K, 340 USD). 2 full sheets of quarter-inch polished plywood at N42, 000: 00K (N84, 000: 00K, 112 USD). A bundle of polyvinyl cellulose ceiling sheets (N18, 000: 00K, 24 USD). One full sheet each of 3-mm and 4-mm plywood at N10, 000: 00K and N12, 500: 00K, respectively (N22, 500: 00K, 30 USD). Two glass cutters at N1500: 00K each (N3, 000: 00K, 4 USD). Ten pieces of 3/4 x 3-inch polished planks at N500 each (N5, 000: 00K, 7 USD). A jar of multipurpose adhesive gum (N18, 000: 00K, 24 USD). 10 packets of adhesive 4-minute adhesives at N450 each (N4, 500: 00K, 6 USD). 20 lengths of ¾-inch pipes at N1700: 00K (N34, 000: 00K), 8 lengths of 1x3 mm flat bar at N2, 800: 00K each (N22, 400: 00K, 30 USD), 10 lengths of 1X3 Angle iron at N2000:00K (N20, 000: 00K, 27 USD), 4 lengths of 4x4 mm rod at N1500 (N6, 000: 00K, 8 USD) and 6 lengths of ¼-inch rod at N1500: 00K (N9000: 00K, 12 USD). 10 tins of black/white car paint at N4, 000: 00K (40, 000: 00K, 53 USD), 2 gallons of Anak paint at N9, 000: 00K (N18, 000: 00K, 24 USD), 4 masking tape at 750 each (N3, 000: 00K, 4 USD). Two soldering devices at N2, 500: 00K (N5,000: 00K, 7 USD), 1 measuring tape (N2500: 00K, 3.5 USD), nails of different sizes (N3, 500: 00K, 4.6 USD), sandpaper (N1, 500: 00K, 2 USD) and 1 metal file (N1, 400: 00K, 2 USD). Welding labour cost (N85, 000: 00K, 113 USD), material transportation and fabrication assistant costs for three weeks (N75, 000: 00K, 100 USD).

2.2 Construction Processes

2.2.1 Light-dark maze

The construction of the starting point (LDM) of the intended mouse anxiety triple test was initiated by cutting 60 x 40 cm pieces out of the polished ¾-inch thick white surfaced plywood (Figs. 1,2,&3). Then the sheets of 3-mm plastic glass were next cut into 60 x 30, 40 x 30, and 40 by 28 cm pieces. Next, the lower edges of the plastic glass pieces were roughed with sandpaper and, with the aid of multipurpose and 4-minute adhesive pastes applied to the roughened surfaces, the plastic glass pieces were fastened along the lengths and breadths of the 60 x 40 cm ¾-inch thick plywood floors in such a way that a 40 x 28 cm glass piece was inserted at the 40 cm point of the 60 cm long floor to effectively partition the LDM into the two parts intended to be 40 x 40 cm light and 20 x 40 cm dark portions (see Fig. 1). The fastened plastic glass pieces were allowed to dry and adhere under applied pressure. The floor of each LDM was divided into sixteen 10 x 10 cm

squares by gridlines using a cutter. A removable wooden roof cap made from ¾ x 3 cm polished wood and 3-mm plywood was made to cover the dark portion of the maze. A 6 x 5 cm aperture was made at the centre of the middle and last partitions of each LDM to facilitate physical connections with the next behavioural apparatus in the intended triple test. An elevated metal platform measuring 41 x 61 cm and 70 cm high was constructed to house the finished LDM. Finally, the light portion, including the floor were painted white while the dark portion with its roof cap was painted black. (Fig. 1).

2.2.2 The elevated zero-maze

The construction of this maze was initiated by cutting pieces of five (5) cm wide ¾ inch thick circular runways with a 160-cm outer and 120-cm inner circumference were made from piled polyvinyl cellulose ceiling sheets (Fig. 1 & 2). Next, the 40 x 18 cm outer and 30 x 18 cm inner walls of the intended elevated zero-maze (EZM) were cut out of the 2-mm plastic glass material. These were each carefully cast into the desired curvatures by semi-melting them at an appropriate distance over a bursen burner flame. The lower edges of the casts were roughened with sandpaper and were, with the aid of the adhesive gums, fasted to the edges of the circular way in way that the walled and unwalled spaces were equal (Fig. 3). A 6 x 5 cm aperture was made at the centre of the lower portion of each outer wall in such a way the first aperture will overlap with the aperture in the LDM and the second aperture will overlap with the aperture of the third component (marble burying maze, MBM) of the intended triple test apparatus. A 70 cm high elevated metal platform was also created to carry the finished EZM. Finally, the walled portions were painted black and the unwalled portions painted white.

2.2.3 The marble burying maze

This was made by fastening roughened lower edges of 40 x 30 cm 3-mm plastic glass plates to the sides of 40 x 40 cm ¾-inch thick, white-surfaced polished plywood pieces by the aid of adhesives gums (Fig. 1 & 2). A 6 x 5 cm aperture was created at the centres of the lower edges of one side of the marble burying device to overlap with the aperture in one of the outer walls of the EZM. The floors of the MBMs were filled with wooden shavings on which 8 pieces of broken marble were placed. An elevated metal platform measuring 41 x 41 cm was also made to house the MBM. The maze with it floor was painted white.

A one-cm thick 2-cm wide layer of polyurethane (mattress) foam was fastened to the edges of the outer surfaces of the apertures to obliterate any gap around them between two adjoining tools in the triple device as well as afford free-flowing inter-tool movements of mice when the axes of the constituent mazes are perfectly aligned.

The designs of metal platforms supporting of the three apparatuses avoided permanent welding of their different parts so that they can be collapsed to manage laboratory space when not in use.

3. RESULTS

Six sets of novel mouse anxiety triple apparatuses (Fig. 1) - each comprising an elevated light-dark, zero-, and marble burying maze - were generated at a total cost of N936, 400: 00K (≈1000 USD) or about N156, 000 (≈ 200 USD) per set.

4. DISCUSSION

One of the ways of overcoming the lull in anxiolytic drug discovery over the past few decades is modification/refinement of the existing behavioural anxiety apparatuses to increase the functional versatility and animal ethics friendliness of their preclinical anxiety assays/models. It is the vision of this study to devise a composite rodent anxiety test paradigm that will not only exhibit robust sensitivity to anxiety-related indices but will also be devoid of the liabilities of earlier multi-tests or their component test tools as much as possible. The novel mouse anxiety triple tool being proposed is the first of its kind to combine elevated light-dark (ELDM), elevated zero- (EZM), and elevated marble-burying (EMBM) mazes - three of the most reliable classical anxiety tests in one simultaneous experimental protocol. The mouse anxiety triple tool appears poised to enjoy the trappings of an improvisation over similar existing animal anxiety test tools on one hand, and of a purpose-driven appropriate technology on the other. The multi-test seems equipped to meet the reduction and refinement components of the now widely accepted 3Rs principles put forward by Bill Russell and Rex Burch over six-decades ago to emphasise the imperativeness of balancing animal ethics with maximizing research data output using the minimal number of animals practically feasible [20,21]. The design of this novel multi-test, just like the triple rodent anxiety

protocol by Ramos (2008), affords a quasi-simultaneous exposure of test animals to the three components this composite tool thus causing a drastic reduction by two-thirds in the number of experimental animals that would have been needed for equal number of trials were they to be carried out on the three constituent tools individually.

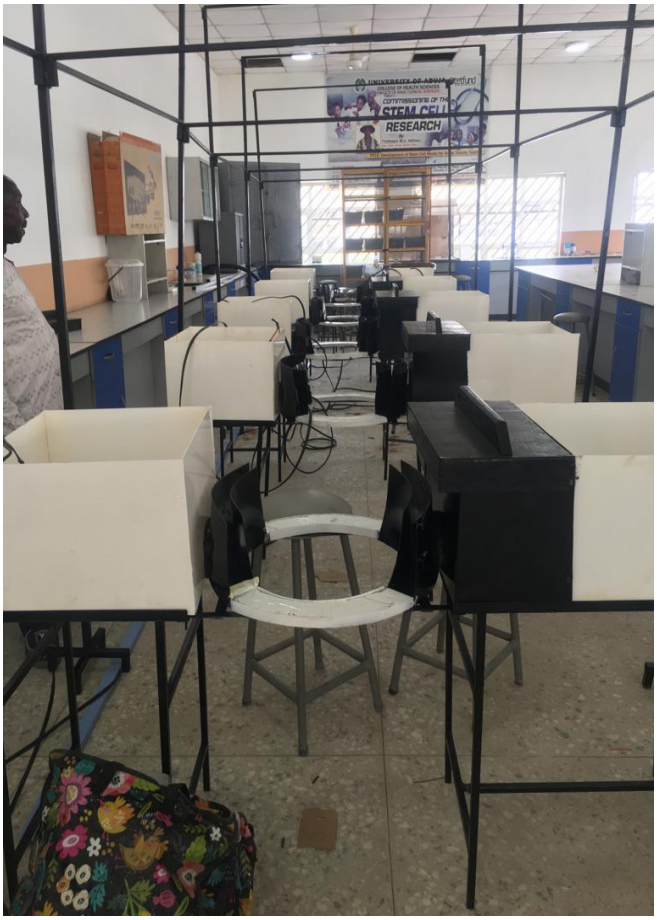

Fig. 1. Sets of U. of A. mouse anxiety triple test apparatus

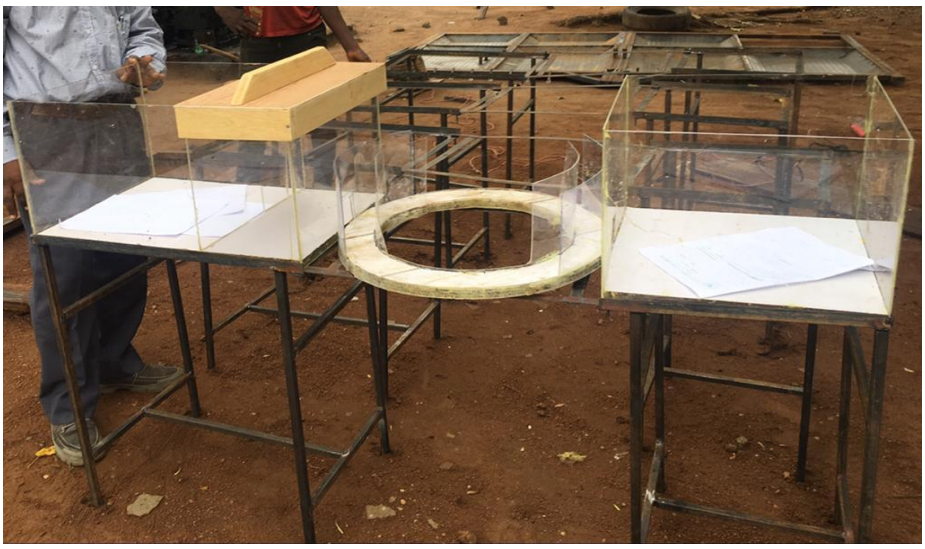

Fig. 2. A set of the U of A mouse anxiety triple test tool (in construction)

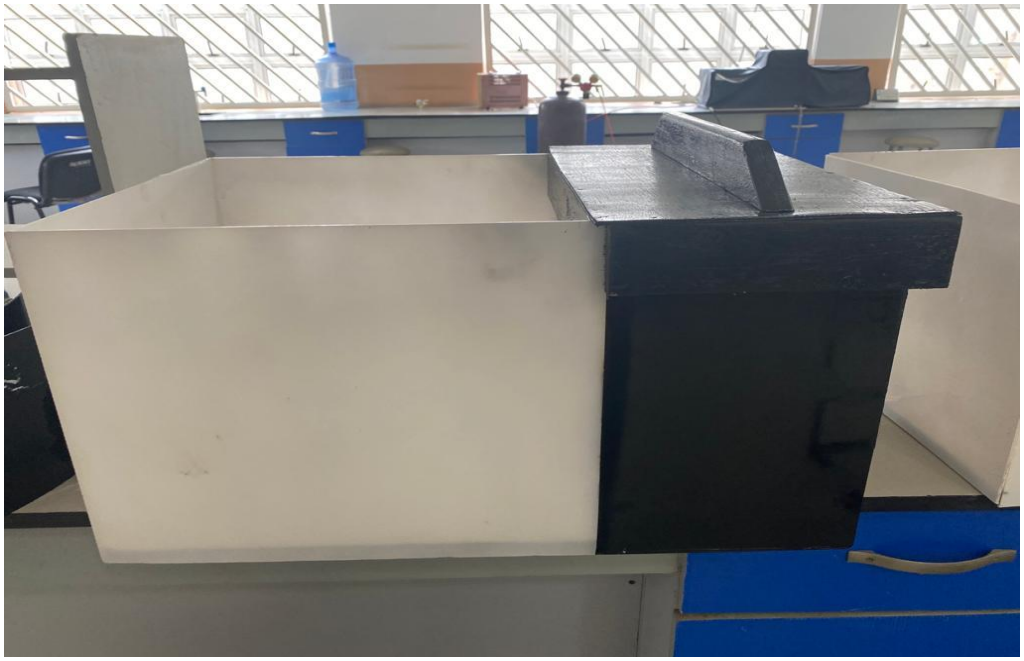

Fig. 3. A unit of light-dark maze

Refinement in the context of this study implies upscaling the efficiency and limiting factors that could potentially have negative impact on the data generated by the novel multi-test. In this sense, both the replacement of the plus-maze in the middle of the design of Ramos (2008) by a zero-maze which clearly eliminates the plus maze's neutral central square along with its ambiguity on behavioural data and that of the open-field's boring environment by a shavings and marbles enriched environment of the MBM should be seen a refinement to existing similar paradigm that will not only broaden the scope of its anxiety sensitivity capacity but will also likely enhance and broaden reproducibility, validity and translational value of its behaioural data output as previously reported [13-19]. Initial ethological evaluations on this novel mouse anxiety tool indicate it can generate anxiety-related behaviours in mice with appropriate time distribution among its open (aversive) and closed (safe) portions. The triple apparatus also exhibits the potential for through put as most mice traversed and explored all the component tools within 22-minute periods of the trials.

High premium is often placed on an appropriate technological device that is cost-effective relative to its local environment [22,23]. Compared to the cost of a set of its foreign counterparts (mouse elevated zero-maze - Stoelting 60190 - $3,020; mouse open-field maze - Stoelting 60100 - $1, 286; and assuming mouse marble-burying maze - $1, 280) at about $5,500 or N4, 125, 000:00k, that of a set of the mouse anxiety triple of this report will cost less than - $200 (less than 4%). The 6 sets of the triple test apparatus that were locally produced from local resource at less than $1,000 would have required about $33,000 or N24, 750, 000:00k to have similar foreign counterparts imported. This is beside the costs of shipping and of assembling them into composite anxiety apparatus since this novelty, within the limits of our literature search, has not been in existence and an order must be placed for it to be supplied at a much later date.

Another positive feature of this new triple test is the fact that there was need for sophisticated machinery in its fabrications - simple tools such as saws, cutters, hammers, paint sprayers, metal files and a medium-size room space were all that were needed. Again, the apparatus and the entire experimental protocol are simple to operate and can be easily set-up. The trials can be carried out by personnel with minimal training; once the experimental animal is gently placed in the first component of the multi-test, the rest of the trial can be completed with no human interference. The mouse anxiety triple assay - including its metal stands - can be set up and dissembled after use within ten minutes; can be easily transferred or be kept at a corner of the laboratory to conserve space.

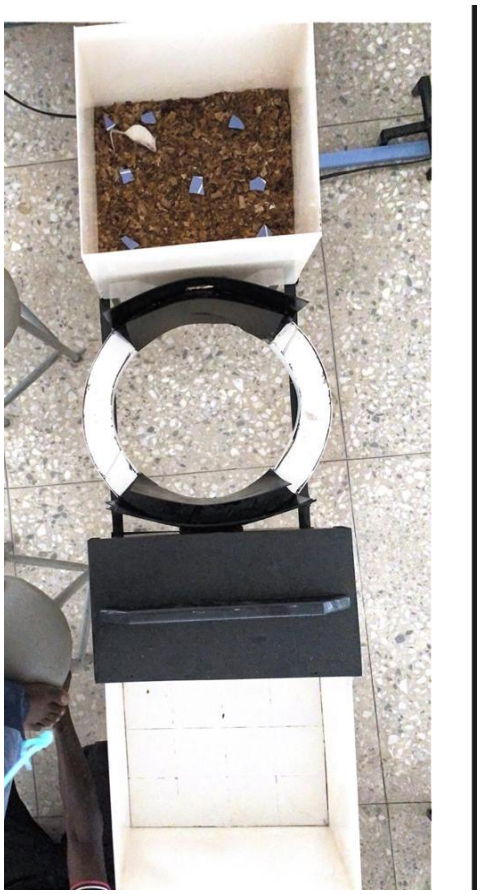

Fig. 4. Aerial view of the triple test with mouse in the marble burying maze

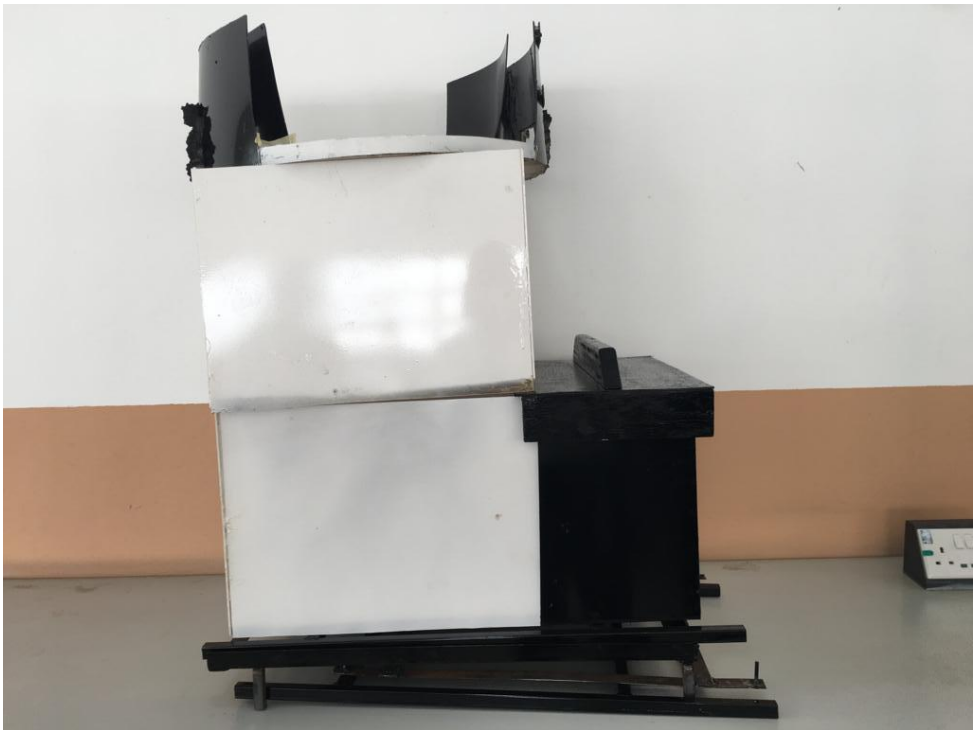

Fig. 5. Collapsed components of the triple test apparatus

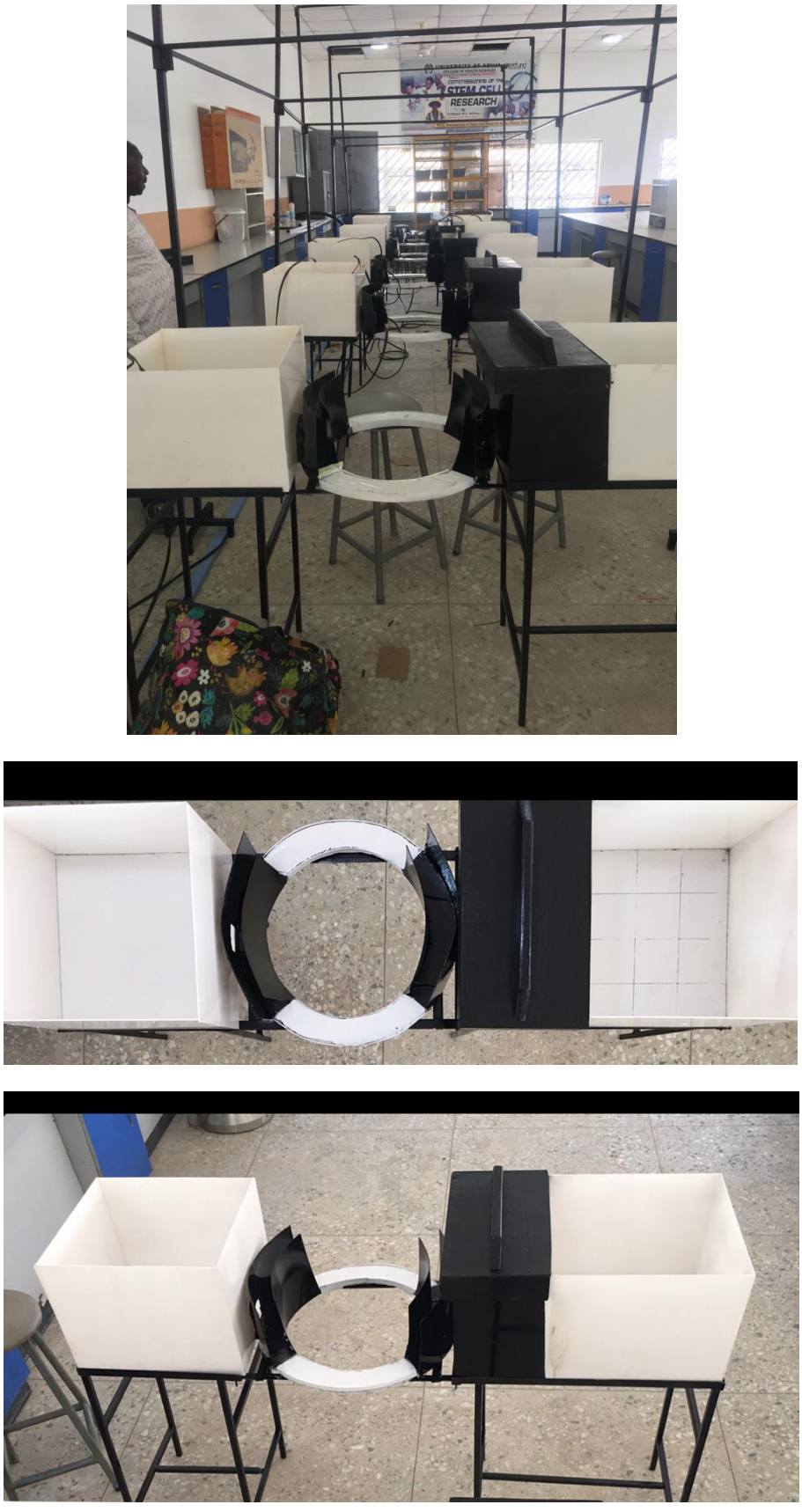

Fig. 6. Side view of the mouse anxiety triple apparatus

Other strengths include the fact that the fabrication is a sustainable venture since all the resources are locally available and comparatively affordable; it poses no health or environmental risks as most parts are made of materials that are inert and non-toxic. These properties are desirable for appropriate technology inventions to achieve sustainability [24,25].

Despite the above highlighted desirable properties of this novel multi-test, the lack of automatic recording of distances travelled by mice is a present drawback to the device and the multi-test. Efforts to overcome this challenge is ongoing since automatic audio-visual recording and transmission of behavioural activities is already in place.

5. CONCLUSION

The novel U. of A. mouse anxiety multi-test is a cost-effective, safe, and environmentally safe innovation that has the potential to enhance preclinical research in anxiolytic drug discovery. However, there is the need for its automation and further pharmacological evaluation.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

1. Javaid SF, Hashim IJ, Hashim MJ, Stip E, Samad MA, Ahbabi AA. Epidemiology of anxiety disorders: Global burden and sociodemographic associations. Middle East Current Psychiatry. 2023;30(1):44.
2. Griebel G, Holmes A. 50 years of hurdles and hope in anxiolytic drug discovery. Nature Reviews Drug Discovery. 2013;12(9):667-687.
3. Leucht S, Heres S, Davis JM. Considerations about the efficacy of psychopharmacological drugs. Der Nervenarzt. 2011;82:1425-1430.
4. Seyhan AA. Lost in translation: The valley of death across preclinical and clinical divide–identification of problems and overcoming obstacles. Translational Medicine Communications. 2019;4(1):1-19.
5. Nicholson JR, Sommer B. The research domain criteria framework in drug discovery for neuropsychiatric diseases: Focus on negative valence. Brain and Neuroscience Advances. 2018;2: 2398212818804030.
6. Holmes A. Targeted gene mutation approaches to the study of anxiety-like behavior in mice. Neuroscience and Biobehavioral Reviews. 2001;25(3):261-273.
7. McIlwain KL, Merriweather MY, Yuva-Paylor LA, Paylor R. The use of behavioral test batteries: Effects of training history. Physiology and behavior. 2001;73(5):705-717.
8. Van Gaalen MM, Steckler T. Behavioural analysis of four mouse strains in an anxiety test battery. Behavioural Brain Research. 2000;115(1):95-106.
9. Himanshu, Dharmila, Sarkar D, Nutan. A review of behavioral tests to evaluate different types of anxiety and anti-anxiety effects. Clinical psychopharmacology and neuroscience: The official scientific journal of the Korean College of Neuropsychopharmacology. 2020;18(3): 341–351.
10. Stukalin Y, Einat H. Analyzing test batteries in animal models of psychopathology with multivariate analysis of variance (MANOVA): One possible approach to increase external validity. Pharmacol Biochem Behav. 2019;178:51-55.
11. Umarudeen AM, Magaji MG, Bello OS, Aminu C, Abdullahi MI. Pharmacological investigation of serial anxiety tests in the mouse: A pilot study. Journal of Advances in Medical and Pharmaceutical Sciences. 2020:25-31.
12. Ramos A, Pereira E, Martins GC, Wehrmeister TD, Izídio GS. Integrating the open field, elevated plus maze and light/dark box to assess different types of emotional behaviors in one single trial. Behavioural Brain Research. 2008;193(2): 277–288.
13. Fraser LM, Brown RE, Hussin A, Fontana M, Whittaker A, O'Leary TP, Lederle L, Holmes A, Ramos A. Measuring anxiety- and locomotion-related behaviours in mice: A new way of using old tests. Psychopharmacology (Berl). 2010;11(1): 99-112.
14. Braun AA, Skelton MR, Vorhees CV, Williams MT. Comparison of the elevated plus and elevated zero mazes in treated and untreated male sprague–dawley rats: Effects of anxiolytic and anxiogenic agents. Pharmacology Biochemistry and Behavior. 2011;97(3):406-415.
15. Tucker LB, McCabe JT. Behavior of male and female C57BL/6J mice is more

consistent with repeated trials in the elevated zero maze than in the elevated plus maze. Frontiers in Behavioral Neuroscience. 2017;11:13.
16. Schneider P, Ho YJ, Spanagel R, Pawlak CR. A novel elevated plus-maze procedure to avoid the one-trial tolerance problem. Frontiers in Behavioral Neuroscience. 2011;5:43.
17. Gulinello M. Behavioral core protocols and training. Behav Core Facility. 2020;4:56.
18. Albelda N, Joel D. Animal models of obsessive-compulsive disorder: exploring pharmacology and neural substrates. Neuroscience & Biobehavioral Reviews. 2012;36(1):47-63.
19. Deacon RM. Digging and marble burying in mice: simple methods for in vivo identification of biological impacts. Nature Protocols. 2006;1(1):122-124.
20. Russell WMS, Burch RL. The principles of humane experimental technique. Methuen; 1959.
21. Goldberg AM. The principles of humane experimental technique: Is it relevant today?. ALTEX - Alternatives to Animal Experimentation. 2010;27(2):149–151.
DOI: 10.14573/altex.2010.2.149
22. Hazeltine B, Bull C. Appropriate Technology; Tools, Choices, and Implications. Academic Press. Inc; 1998.
23. Bonhomme M. Appropriate Technology: Tools, Choices, and Implications. ASEE Prism. 1999;9(4):32.
24. Sianipar C, Dowaki K, Yudoko G, Adhiutama A. Seven pillars of survivability: Appropriate technology with a human face. European Journal of Sustainable Development. 2013;2(4):1-18.
25. Sianipar CPM, Dowaki K, Yudoko G. Environmental impacts of Appropriate Technology: The system boundaries. Advanced Science, Engineering and Medicine. 2014;6(1):141-142.

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