ISSN 0974-3618 (Print)
0974-360X (Online)

www.rjptonline.org

RESEARCH ARTICLE

An Apparatus for the determination of rat paw Edema during *In vivo* Evaluation of Anti-inflammatory agents

U. S. Jijith[1,2], S. Jayakumari[1]

[1]School of Pharmaceutical Sciences, Vels Institute of Sciences, Technology and Advanced Studies (VISTAS), Pallavaram, Chennai-600117, India

[2]College of Pharmaceutical Sciences, Govt. Medical College, Kozhikode-673008, Kerala, India

*Corresponding Author E-mail: **nisajayaa@gmail.com**

ABSTRACT:

In vivo evaluation of anti-inflammatory agents normally involves the measurement of rat paw volume. Some notable equipment for *in vivo* measurement of rat paw volume includes plethysmometer, screw gauge and vernier calipers. Balance method is also reported for the purpose. The present study describes development and validation of a simple apparatus for the determination of rat paw volume suitable for *in vivo* evaluation of anti-inflammatory agents.The calibration plot obtained with the developed apparatus was linear with a high R^2 value of 0.983. Thus it was confirmed that the apparatus is useful for the determination of rat paw volume.

KEYWORDS: Paw edema, plethysmometer, screw gauge, vernier calipers, balance method.

1. INTRODUCTION:

Evaluation of anti-inflammatory activity is a common requirement in development of novel therapeutic agents against inflammation [1-6]. A good number of methods have been suggested for determination of rat paw edema during *in vivo* evaluation of anti-inflammatory agents [7-10]. Some notable equipment includes plethysmometer [11], screw gauge [12] and vernier calipers [13]. Balance method is also reported. Among these, the plethysmometer enjoys a prime position in its acceptability for measuring paw edema [14,15]. Modified apparatus are also reported [16]. It has already been reported that measurement of raw paw edema is always prone to error. In order to develop a cheap, reliable and simple laboratory method, we have developed an apparatus for the determination of rat paw edema.

Received on 19.06.2019 Modified on 28.07.2019
Accepted on 13.09.2019
Research J. Pharm. and Tech 2020; 13(5):2373-2375.
DOI: 10.5958/0974-360X.2020.00426.6

2. MATERIALS AND METHODS:

Materials:

Purified water was used in the study.

Design and development of an apparatus for determination of rat paw volume:

In order to develop a cheap, reliable and simple laboratory method, we have developed an apparatus for the determination of rat paw edema. The instrument works on the principle of displacement of water on immersion of rat paw into a chamber filled with water [17,18].

Working of the apparatus:

The apparatus consists of a T-tube. One of the openings of the main tube (not the opening of the side tube) of the T-tube is closed and that part is firmly fixed onto a heavy base. The fixing of the T-tube to the heavy base provides sufficient stability to the apparatus during its operation. The position of the T-tube was in such a way that the side tube with opening faces to one side of the apparatus. The other end of the main tube was faced upward and allows entry of rat paw. The side tube is fitted with a side pipe. When water is added into the apparatus it gets filled into the apparatus until the level reaches the point of attachment of the side pipe. Now after filling the apparatus with water, if we add some more water it will flow outwards through the side tube.

Interestingly, the volume expelled through the side tube would be equal to the volume of water added. Thus we presumed that if we could immerse the rat paw into the apparatus pre-filled with water, it would displace water. Moreover, the volume of water displaced by the paw would be equal to its volume. The larger diameter of the main tube provides easy visibility and immersion of rat paw through it.

Calibration of the apparatus:

In order to ensure that the developed apparatus works properly and accurately, we carried out calibration of the apparatus. The calibration was carried out by addition of standard volume of water into the prefilled chamber of the apparatus. The water displaced through the outlet was collected into a pre-weighed microcentrifuge tube. The final weight of the microcentrifuge tube with displaced volume of water was determined. The density of the water used was also noted. Using the weight of displaced water collected in the centrifuge tube and the density of water, the volume of displaced water was determined.

3. RESULTS AND DISCUSSION:

The developed apparatus is shown in Figure 1.

Figure 1: Developed apparatus for determination of paw volume

Calibration of the apparatus:

To the pre-filled apparatus, different volumes of water (0.05, 0.1, 0.2, 0.3, 0.4, 0.5, 0.6, 0.7, 0.8, 0.9 and 1.0 mL) were added. Each time the displaced quantity of water was collected into a pre-weighed microcentrifuge tube. The volume of displaced water was determined (Table 1). The experiment was carried out in triplicate. Figure 2 shows the calibration plot obtained with the developed apparatus. The obtained plot was linear with a high R^2 value of 0.983. Thus it was confirmed that the apparatus is useful for the determination of volume.

Table 1: Data of calibration plot

Volume of water added (mL)	Volume of determined by the developed method ± SD (mL)
0.05	0.180±0.047
0.1	0.208±0.035
0.2	0.253±0.045
0.3	0.337±0.020
0.4	0.447±0.105
0.5	0.587±0.007
0.6	0.682±0.073
0.7	0.671±0.048
0.8	0.776±0.067
0.9	0.872±0.078
1.0	1.033±0.045

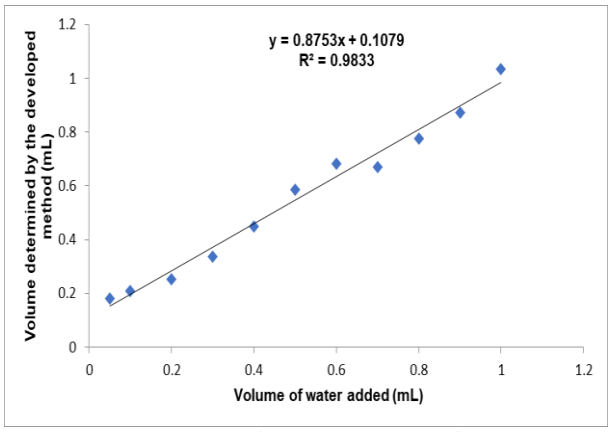

Figure 2: Calibration plot of water displacement for the developed apparatus

4. SUMMARY AND CONCLUSIONS:

A new apparatus was developed for the determination of paw volume of rats. The calibration plot obtained with the developed apparatus was linear with a high R^2 value of 0.983. Thus it was confirmed that the apparatus is useful for the determination of rat paw volume.

5. CONFLICT OF INTEREST:

USJ has made a patent application (Indian Patent Application No. 201941009319 dated 11th March 2019) related to this work.

6. REFERENCES:

1. Sugumaran M, Vetrichelvan T, Quine SD. Anti-inflammatory activity of Folklore: Pithecellobium dulce Benth. Research J. Pharm. and Tech.2 (4): 2009; 868-869.
2. Gupta V, Bansal P, Kumar P, Kaur G. Anti-Inflammatory and Anti-Nociceptive Activity of *Adiantum capillus*. Research J. Pharm. and Tech. 3(2): 2010; 432-434.
3. Karishma Sk, Lakshmi K, Tony DE, Babu AN, Nadendla RR. Pharmacological Evaluation of Leaf Extract of Terminalia bellerica with Moringa oleifera for its Synergistic Action on Anti-diabetic Activity and Anti-inflammatory Activity in Rats. Research J. Pharm. and Tech. 2019; 12(3): 1181-1184.
4. Dhalendra G, Satapathy T, Roy A. Animal Models for Inflammation: A Review. Asian J. Pharm. Res. 3(4): 2013; 207-212.
5. Malviya KG, Shivhare UD, Srivastav P, Shivhare SC. Evaluation of Anti-inflammatory Potential of Cyathocline lyrata Cass Plant Extract by using Carrageenan Induced and Formalin Induced Rat Paw Edema. Asian J. Res. Pharm. Sci. 2013; 3(2):90-94.
6. Sam SG, Kumar A, Babu DV, Swamy VBN. Evaluation of Anti-diabetic and Anti-inflammatory activities of ethanolic extract of whole plant of Inula racemosa. Research Journal of Pharmacology and Pharmacodynamics. 2015; 7(3): 129-134.
7. Evans PD, Hossack M,Thomson DS. Inhibition of contact sensitivity in the mouse by topical application of corticosteroids. Br J Pharmacol 1971; 43: 403.
8. Tonelli G, Thibault L and Ringler I. A bio-assay for the concomitant assessment of the antiphlogistic and thymolytic activities. Endocrinology 1965; 77: 625–630.
9. Chang J, Blazek E, Skowronek M, Marinari L, Carlson RP. The antiinflammatory action of guanabenz is mediated through 5-lipoxygenase and cyclooxygenase inhibition . Eur J Pharm 1987; 142: 197–205.
10. DeYoung LM. The mouse ear inflammatory response to topical arachidonic acid. J Invest Dermatol 1984; 82: 367–371.
11. Sehgal R, Kumar, VL, Calotropisprocera Latex-Induced Inflammatory Hyperalgesia—Effect of Antiinflammatory Drugs. Mediators of Inflammation, 2005(4), 216–220.
12. Singh S, Kumar R, Jain H, Gupta YK. Anti-inflammatory and antiarthritic activity of UNIM-301 (a polyherbalunani formulation) in Wistar rats.Pharmacognosy Research, 2015; 7(2), 188–192.
13. Sarkhel S. Evaluation of the anti-inflammatory activities of *Quillajasaponaria*Mol.saponin extract in mice. Toxicology Reports. 2016;3:1-3.
14. Sarkar M.Evaluation of the Anti-Inflammatory Activity of Methanolic Leaf Extract of Mangiferaindica L. (Anacardiaceae) in Rats. Int J Drug Dev& Res 2015, 7(3),21-25
15. Srebro D, Vučković S, Milovanović A, SavićVujović K, Prostran M .Evaluation of Prophylactic and Therapeutic Effects of Tramadol and Tramadol Plus Magnesium Sulfate in an Acute Inflammatory Model of Pain and Edema in Rats.Front Pharmacol. 2018 16(9)1326.
16. Shejawal N, Menon S, Shailajan S. A simple, sensitive and accurate method for rat paw volume measurement and its expediency in preclinical animal studies. Hum Exp Toxicol. 2014 Feb;33(2):123-9.
17. DolanM G. Effects of Cold Water Immersion on Edema Formation After Blunt Injury to the Hind Limbs of Rats.J Athl Train. 1997 Jul; 32(3): 233–237
18. Dolan MG, Mychaskiw AM, Mendel FC. Cool-Water Immersion and High-Voltage Electric Stimulation Curb Edema Formation in Rats.J Athl Train. 2003;38(3):225-230.