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Study of analgesic and Anti-inflammatory activity of of *Murraya koenigii* on albino wistar rats

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ABSTRACT

Murraya koenigii (Linn) is a plant of the Rutaceae family, well known in India as curry leaves. In the present study, evaluation of physicochemical characteristics; preliminary phytochemical parameters and pharmacological activities of methanol extract of dried leaves of *Murraya koenigii* Linn has been carried out. The preliminary phytochemical studies of the methanol extract of the leaves showed the presence of various phytochemical constituents such as alkaloids, tannins, flavonoids, steroids and saponins. . The aim of the present study was carried out with the objective of phytochemical screening and to evaluate the antiinflammatory, analgesic activity methanol extract of *Murraya koenigii* Linn Extract was studied for antiinflammatory activity by using carrageenan-induced hind paw edema in albino rats and the mean increase in paw volume and % inhibition in paw volume were measured plethysmometrically at different time intervals after carrageenan (1% w/v) injection. Extract was also evaluated for analgesic activity using Eddy's hot plate method and acetic acid-induced writhing method in albino rats. The methanol extract showed significant ($P < 0.001$) reduction in the carrageenan -induced paw edema and analgesic activity evidenced by increase in the reaction time by eddy's hot plate method and significantly reduced ($P<0.001$) acetic acid induced writhing. The methanol extract showed antiinflammatory and analgesic effect in dose dependent manner when compared with the control and standard drug, diclofenac sodium (10mg/kg, p.o). These inhibitions were statistically significant ($P < 0.01$). Thus our investigation suggests a potential benefit of *Murraya koenigii* Linn in treating conditions associated with inflammatory pain.

Keywords: *Murraya koenigii,* Analgesic activity, Eddy's hot plate, Plethysmometrically, Acetic acid induced writhing

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INTRODUCTION

Plants have been used in treating human diseases for thousands of years. Some 60,000 years ago, it appears that Neanderthal man valued herbs as medicinal agents; this conclusion is based on a grave in Iran in which pollen grains of eight medicinal plants were found. *Murraya koenigii. Linn*, commonly known as curry leaf or kari patta in Indian dialects, belonging to Family Rutaceae which represent more than 150 genera and 1600 species (1). It is an important export commondity from India as it fetches good foreign revenue. A number of chemical constituents from every part of the plant have been extracted. The most important chemical constitutents responsible for its intense characteristic aroma are Pgurjunene, P-caryophyllene, P-elemene and O-phellandrene. *M. koenigii.* is widely used in Indian cookery for centuries and have a versatile role to play in traditional medicine.(2) The plant is credited with tonic and stomachic properties. Bark and roots are used as stimulant and externally to cure eruptions and bites of poisonous animals. Green leaves are eaten raw for cure of dysentery, diarrhoea and for checking vomiting. Leaves and roots are also used traditionally as bitter, anthelmintic, analgesic, curing piles, inflammation, itching and are useful in leucoderma and blood disorders. Several systematic scientific studies are also being conducted regarding the efficacy of whole plant or its parts in different extract forms for the treatment of different diseases (3). *M. koenigii* contains a number of chemical constituents that interact in a complex way to elicit their pharmacodynamic response. This plant has been reported to have anti-oxidative, cytotoxic, antimicrobial, antibacterial, anti ulcer, positive inotropic and cholesterol reducing activities. (4) Curry leaf trees are naturalized in forests and waste land throughout the Indian subcontinent except in the higher parts of the Himalayas. The plants were spread to Malaysia, South Africa and Réunion Island with South Asian immigrants (5). Bark and the roots are used as a stimulant by the physicians. They are also used externally to cure eruptions and the bites of poisonous animals. It has Vitamin A, Vitamin B, Vitamin C, Vitamin B2, Calcium and iron in plenty. Curry leaves come handy in treating such conditions. A paste made of curry leaves is applied on these persistent boils for quick relief. Along with mint leaves and coriander leaves, curry leaves can be used in treating excessive pitta conditions. (7) Kidney pain can be cured by using juice of root of *Murrayakoenigii*. *Linn.* Inflammation results in the liberation of endogenous mediators like histamine, serotonin, bradykinin, prostaglandins etc (8). These mediators even in small quantities can elicit pain response. Based on phytochemical investigation presence of saponins and flavonoids are the constituents which are used for and other complication by providing anti-

inflammatory activity (10). For centuries, medicinal plants are the basis for the treatment of various diseases. Nearly 80% of people living in developing countries still depend on plant-based traditional medicine for their primary health care and almost three-fourths of the herbal drugs used worldwide are derived from medicinal plants. Based on phytochemical screening of *Murraya koenigii*. *Linn* leaves of methanolic extract shows the presence of some flavonoids and terpenoids are used for the investigation of analgesic activity hot-plate method and acetic acid induced writhing method (9).

MATERIALS AND METHOD

The designing of methodology involves a series of steps taken in a systematic way in order to achieve the set goal(s) under the prescribed guidelines and recommendations. It includes in it all the steps from field trip to the observation including collection of the drugs and chemicals, plant material collection, preparation of plant extracts, preliminary phytochemical analysis of the extracts, selection of dose for animal study. Healthy adult albino wistar rats weighing 200-250grams of either sex were selected for the study. Animals were housed in appropriate cages in uniform hygienic conditions and fed with standard pellet diet (Amrul Laboratory Animal Diet) and water ad libitum. They were fasted overnight before the day of experiment, after 72hours of fasting from the day of Alloxan introduction. Animals were housed within the departmental animal house and the room temperature was maintained at 27° C. Animal studies had approval of IAEC. The leaves of *Murraya koenigii*. *Linn* was collected from the Geethanjali College in the month of October and was identified and authenticated from Department of pharmacognosy GCOP. The fresh plant material collected was thoroughly cleaned by washing under running tap water and air-dried in shade for seven days. It was then homogenized to fine powder and stored in air-tight bottles for further studies. 51 grams of dried leave powder of *Murraya koenigii Linn* was successively extracted by Soxhlet extraction method using methanol as solvent. The solvent was evaporated under reduced pressure and the extract thus obtained was stored in air-tight bottles at 4ºC. The methanolic extract of *Murraya koenigii* Linn suspended in water. All the drugs were administered i.p for experimental purpose. Each time preparations of the extracts were prepared when required. The drugs were administered at a constant volume of 10ml/kg for each animal. Rats were divided into four groups (n=6). Acute inflammation was produced by sub planter administration of 0.1 ml of 1% w/v carrageenan in normal saline in the right hand paw of the rats. The paw volume was measured at 0-h and 3-h after carrageenan injection by using plethysmometer. Animals of group I received normal saline (3 ml/kg b.w., intraperitoneal, i.p)

and served as saline control. The group II received reference drug Diclofenac sodium(10 mg/kg b. w., i.p).The groups III and IV received methanol extract of *Murraya koenigii Linn* (200 and 400 mg/kg b.w., i.p, respectively) and Animals of all groups were treated with the extract and reference drug 1 hour before the administration of carrageenan. (12, 13, 14) The difference between the intial and subsequent readings gave the actual edema volume Edema was expressed as the mean increase in paw volume relative to control animals. For Hot plate method, Male Sprague-Dawley mice (40-50 g) were divided into four groups containing of 6 animals of each group-1 received normal saline (3 ml/kg b.w., intraperitoneal, i.p), The groups II and III received methanol extract of Murraya koenigii *Linn* (200 and 400 mg/kg b.w., i.p) and group IV received reference drug Diclofenac sodium (10 mg/kg b. w., i.p) and they were housed under standard conditions of temperature (25±2ºC) and 12 hr/12 hr light/dark cycles. The animals were kept under laboratory conditions for one week before start of the experiments and allowed food and water *ad libitum*. Take the basal reaction-time by observing hind paw licking or jumping response in animals when placed on the hot-plate maintained at constant temperature (55^0C). Normally animals showed such response in 6-8 sec. a cut-off period of 15sec i.e. observed to avoid damage to the paws. Inject the standard drug to group-4 and test drugs to group-2&3 and noted the reaction time of animals on the hot plate at 15, 30, 60 and 120min after the drug administration. Calculated the percentage increase in reaction time at each time interval. (21) Acetic acid induced writhing method was adopted for evaluation of analgesic activity. Writhing is defined as a stretch, tension to one side, extension of hind legs, contraction of the abdomen so that the abdomen of mice touches the floor, turning of trunk (twist). Any writhing is considered as a positive response. Swiss albino mice weighing between 15-35g were randomly selected and divided into four groups denoted as group -I, group-II, group-III and group-IV consisting of 4 mice in each group. Each group received a particular treatment i.e. control, standard and the two doses (200 and 400 mg/kg) of the extract. Each mice was weighed properly and the dose of the test samples and control materials were adjusted accordingly. The analgesic activity of the samples was evaluated using acetic acid induced writhing method in mice. (24) In this method, acetic acid is administered intraperitoneally to the experimental animals to create pain sensation. As a positive control, any standard NSAID drug can be used. In the present study Diclofenac sodium was used to serve the purpose. The plant extract was administered orally in two different doses (200 and 400 mg/kg body weight) to the Swiss Albino mice after an overnight fast. Test samples and vehicle were administered orally 30 minutes prior to intraperitoneal administration of 0.7% v/v acetic acid solution (0.1ml/10g) but Diclofenac sodium was administered 15 minutes

prior to acetic acid injection. Then the animals were placed on an observation table. Each mouse of all groups were observed individually for counting the number of writhing they made in 15 minutes commencing just 5 minutes after the intraperitoneal administration of acetic acid solution. Full writhing was not always accomplished by the animal, because sometimes the animals started to give writhing but they did not complete it. This incomplete writhing was considered as half-writhing. Accordingly, two half-writhing were taken as one full writhing. The number of writhes in each treated group was compared to that of a control group while Diclofenac sodium (10 mg/kg) was used as a reference substance. (25)The results are expressed as means ± S.E.M Differences in mean values of 3 animals in each group were analyzed by a one -way analysis of variance (ANOVA). Statistical significance was assessed as $p < 0.01$.

RESULTS AND DISCUSSION

Phytochemical investigation of aqueous and alcoholic extracts of *Murraya koenigii.Linn* revealed the presence of alkaloids, tannins, saponins, steroids, Anthracene glycosides and flavonoids as secondary metabolites. In carragenan induced paw oedema activity, the paw volumes and percentage of inhibition of the control, standard and test compounds are shown in Table No: 1. The test compounds are compared with diclofenac as a standard at a dose of 10mg/kg for anti-inflammatory activity. Presently diclofenac showed 20% inhibition of inflammation at 3 hours when compared to control. Alcoholic extracts of *Murraya koenigii Linn* leaves (200 mg/kg and 400 mg/kg) shown significant inhibition of inflammation with 30% and 10% respectively at 3 hours when compared with control. The results of test compounds were found to be statistically significant at valueP<0.01. The analgesic activity of test I and test II was assessed using Hot plate method in Swiss albino rats were illustrated in Table No: 2. Test I and test II showed significant analgesic activity at 200 mg/kg and 400 mg/kg, i.p. Analgesic activity was comparable with the standard drug Diclofenac. Two doses, 200mg/kg and 400 mg/kg showed maximum analgesic activity at reaction time is 12 and 8 sec respectively and the standard drug Diclofinac reaction time is 12 sec. The analgesic activity of test I and test II was assessed using Hot plate method in Swiss albino rats were illustrated in Table No: 3. (21, 22) Test I and test II showed significant analgesic activity at 200 mg/kg and 400 mg/kg, i.p. Analgesic activity was comparable with the standard drug Diclofenac. Two doses, 200mg/kg and 400 mg/kg showed maximum analgesic activity by showing reduction in writhing events of 17 and 9 respectively and the standard drug Diclofinac total writhing events shows 8.

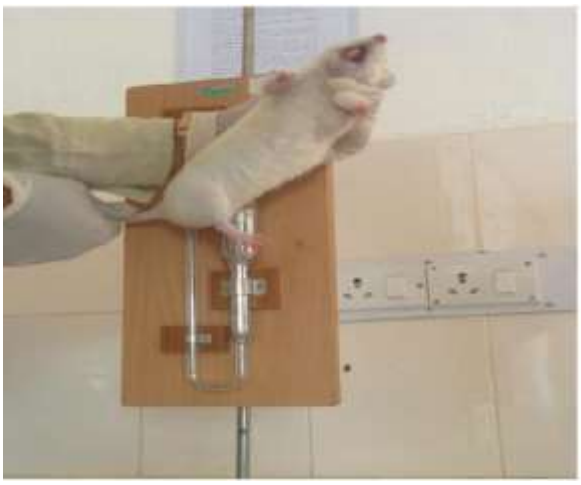

Figure 1: Holding rat while taking reading through plethysmometer.

Figure 2: Measurement of analgesic activity using hot plate method

Figure 3: Holding the rat while administering the drug through

Figure 4: Writhing of the rat after administering 7% acetic acid

Table-1: Effect of extract of *Murraya koenigii Linn* on paw edema volume

Group	Dose (mg/kg)	Change in paw volume (c.c.) Mean±SEM			
		0min	1hr	2hrs	3hrs
Control	-	0.2±0. 01	0.5±0.01	0.5±0.01	0.4±0.01
Standard (Diclofenac Sodium)	10	0.2±0.01	0.3±0.01	0.3±0.01	0.2±0.01
Methanol Extract (Test I)	200	0.3±0.01	0.4±0.02	0.3±0.01	0.3±0.02
Methanol Extract (Test II)	400	0.2±0.01	0.3±0.01	0.2±0.01	0.2±0.01

Table-2: Effect of extracts of *Murraya koenigii. Linn* on Analgesic activity by hot plate method

Groups	Dose (mg/kg)	Basal reaction time (Sec)		Reaction after drug administration (sec)							
		0 min		15 mins		30 mins		60 mins		120 mins	
		Paw licking(P)	Jumping (J)	P	J	P	J	P	J	P	J
Control	-	7±0.1	8±0.3	5±0.2	6±0.1	6±0.3	4±0.1	4±0.3	5±0.1	6±0.3	5±0.1
Standard	10	13±0.3	14±0.1	12±0.1	13±0.3	13±0.2	14±0.1	15±0.2	14±0.1	15±0.2	16±0.1

(Diclofenac Sodium)											
Methanol Extract (Test I)	200	10±0.2	12±0.1	8±0.2	9±0.1	10±0.2	9±0.3	11±0.2	10±0.3	12±0.2	11±0.3
Methanol Extract (Test II)	400	12±0.1	13±0.3	11±0.1	12±0.2	12±0.1	13±0.2	12±0.1	12±0.2	14±0.1	13±0.2

Table-3: Effect of extracts of *Murraya koenigii Linn* on Analgesic activity by acetic acid induced writhing method

Groups	Pretreatment time (min)	Dose injected (ml)	Total Writhing events
Control	60	0.1	22±0.1
Standard (Diclofenac Sodium): 10mg/kg	60	0.1	08±0.2
Methanol extract (200mg/kg)	60	0.4	17±0.2
Methanol extract (400mg/kg)	60	0.4	09±0.1

The results are expressed as means ± S.E.M Differences in mean values between groups were analyzed by a one -way analysis of variance (ANOVA). Statistical significance was assessed as $p < 0.01$.

It is believed that current anti-inflammatory drugs such as opioids and non-steroidal anti-inflammatory drugs are not useful in all cases because of their side effects and low potency. As a result, search for other alternatives became necessary and imperative. Therefore, the present study was aimed at evaluating the scientific basis for the traditional use of *Murraya koenigii Linn* leaves using carragenan induced rat paw edema for anti-inflammatory models. Carrageenan has been widely used as a harmful agent able to induce experimental inflammation for the screening of compounds possessing anti-inflammatory activity. Carrageenan induced rat paw edema is a suitable model to predict the value of anti-inflammatory agents, which act by inhibiting the mediators of acute inflammation. Carrageenan -induced hind paw edema in rat is a biphasic event. The early phase (90 - 180 min) of the inflammation is due to the release of histamine, serotonin and similar substances; and the later phase (270– 360 min) is associated with the activation of kinin -like substances, i.e., prostaglandins, proteases and lysosome. The methanolic extract of *Murraya koenigii Linn* leaves inhibited the carrageenan induced rat paw edema formation, at both early and later phase. This result tends to suggest that the inhibitory effect of the extract on edema formation is probably due to the inhibition of the synthesis and/or release of the inflammatory mediators, especially the cyclooxygenase products. The carrageenan induced paw edema test is effectively controlled with the arachidonate cyclooxygenase (COX) inhib itors due to its COX-dependent mechanism, thus, it is suggested that the MEMK (test I) and MEMK

(test II) may possess arachidonate COX inhibitory property.

The extracts increased reaction latency to thermal pain induced by the hot plate method in rats, which is a specific central antinociceptive test. Inhibition of histamine or kinin pathway may reduce pain. The results of the present study also showed that extract exhibited a comparable magnitude of antinociceptive activity in hot plate method of pain which suggested that the phytochemical constituents are responsible for the analges ic effect. The analgesic activity of some flavonoids and steroids already has been reported suggesting that these or similar constituents may be responsible for the analgesic effect of the extract. The results of the present study indicated that the aqueous and alcoholic extracts of *Murraya koenigii Linn* might contain constituents capable of relieving or modifying responses to pain caused by either thermal or chemical stimulation of the nociceptors mediated by both central and peripheral mechanisms. The acetic acid-induced abdominal contortions or writhing reflex model is a sensitive method for screening analgesic effects of compounds. Some chemicals such as acetic acid could induce abdominal contortions in laboratory animals.The writhing reflex seen in this experiment was produced by injection of 0.6% glacial acetic acid. Intraperitoneal injection of acetic acid produces writhing reflex in the animals by activation of the chemo -sensitive nociceptors. The percent reduction in the number of abdominal contortions indicates the level of analgesia in the acetic acid writhing reflex model. The acetic acid induced abdominal constrictions or writhing was significantly reduced by *Murraya koenigii Linn* extract in both the doses used in a dose dependent manner when compared to the negative group. The analgesic effect produced by the extract at the dose of 400mg/kg was better than the reference drug. Acetic acid induced writhing reflex is sensitive method for screening peripherally acting analgesics and the response in thought to involve local peritoneal cells and mediated through the prostaglandin pathway. This suggests that the analgesic effect of *Murraya koenigii Linn* seen in this study may be mediated through peripheral pain mechanism and or may be through inhibition of the activities or synthesis of prostaglandins .

CONCLUSION

Methanolic extracts of leaves of *Murraya Koenigii.Linn.* have been screened for some pharmacological activities and found to possess anti-diabetic, cholesterol reducing property, anti-diarrhea activity, cytotoxic activity antioxidant property, antiulcer activity antimicrobial, antibacterial potential and many more useful medicinal properties. Among these studies, it could be concluded that leaves of *Murraya Koenigii. Linn* have shown great potential of anti-

inflammatory and Analgesic activity. Based on phytochemical screening, we have concluded that the both tes t I and test II have anti-inflammatory activity in carragenan-induced paw edema in rats. This extracts has showed that decrease in paw edema volume when compared to control and standard drugs. This extracts has showed that increase mean latency to thermal pain and reduced writhing events. The presence of some flavonoids and steroids contains a capable of relieving or modifying responses to pain caused by inhibition of histamine or kinnin pathway. The results indicates that the extract having significant Analgesic and anti Inflammatory activity which is comparable to the standard drug (Diclofenac sodium). If the extract used for isolation of your compound that your compound can be utilized for rational designing of analgesic and anti-inflammatory drugs.

REFERENCES

1. Satyavati G. V., Gupta A. K., Tandon N. Medicinal Plants of India. Vol. 2. New Delhi: Indian Council of Medical Research, India; 1987. pp. 289–299
2. Kokwaro JO. Medicinal Plants of East Africa.1st ed. Nairobi: Kenya East Africa Literature Bureau; 1976.
3. Giday M, Asfaw Z, Woldu Z, Teklehaymanot T. Medicinal plant knowledge of the Bench ethnic group of Ethiopia: an ethnobotanical investigation. Journal of Ethnobiology and Ethnomedicine 2009.
4. Mhaskar KS, Blatter E, Caius JF. Kirtikar and Basu's Illustrated Indian Medicinal Plants Vol. I. XI. 3rd Edn. Indian Medical Science Series # 86-96. Delhi, India; 2000.
5. Narasimhan NS, Paradkar MV, Chitguppi VP, Kelkar SL. Alkaloids of Murraya koenigii: Structures of mahanimbine, koenimbine, - -mahanine, koenine, koenigine, koenidine & + - isomahanimbine. Ind J of Chem 1975; 13: 993-999.
6. Rakesh K Sindhu, Sandeep Arora. Phytochemical and Pharmacognostical Studies on Murraya koenigii L spreng Roots. Drug Invention Today 2012; 4 1 :325-333
7. Kureel SP, Kapil RS, Popli SP. Terpenoid alkaloids from Murraya koenigii Spreng.-II. The constitution of cyclomahanimbine, bicyclomahanimbine & mahanimbidine. Tetrahedrone Letters 1969; 44:3857-3862.
8. Dubois RW, Melmed GY, Laine L, Henning JM. Guidelines for the appropriate use of non-steroidal anti-inflammatory drugs, COX- 2 Specific inhibitors and proton pump inhibitors in patients requiring chronic anti-inflammatory therapy. Ailment Pharmacol. Ther. 2004; 19:197.

9. Hardman JG, Limbird LE, Molinoff PA. Analgesic-antipyretic and anti-inflammatory agents and drugs employed in the treatment of gout., 9th ed., The Pharmacological Basis of Therapeutics, McGraw-Hill, New York. 1996; 617.
10. Wolfe MN, Lichtestein DR, Singh GN. Gastrointestinal Toxicity of Non -Steroidal Anti-inflammatory Drugs. N. Engl. J. Med. 1999; 128.
11. Geissman TA. Flavonoid compounds, tannins, lignins and related compounds. In: Florkin M, Stotz E H, editors. Pyrrole pigments, isoprenoid compounds and phenolic plant constituents.9th ed.New York (N.Y): Elsevier; 1963.p. 265.
12. Schultes R E .The kingdom of plants. In: Thomson W A R, editor. Medicines from the Earth. New York (N.Y): McGraw-Hill Book Co. 1978. p. 208.
13. Babu, S. A. R., and S. S. Karki. "Anti inflammatory activity of various extracts of roots of Calotropis procera against different inflammation models."International journal of pharmacy and pharmaceutical sciences 3.3 (2011): 191-194.
14. Kirkova, M., T. Kassabova, and E. Russanov. "In vivo effects of indomethacin—II. Antioxidant enzymes in metal-deficient rats." General Pharmacology: The Vascular System 23.2 (1992): 151-154.
15. MMJ, Vijay Kumar, B. Eswarappa, and Yadav D. Bodke. "An investigation on anti-inflammatory potency on various extracts of stem bark of *Bauhinia variegata Linn*. by carrageenen induced paw edema method." Journal of Natural Product & Plant Resources 4.3 (2014).
16. Ajitha, M. "Synthesis and evaluation of new 3-substituted-[3, 4-dihydro pyrimidinones]-indolin-2-ones for anti inflammatory activity m. ajitha and k. rajnarayana2."
17. RM, Rohini, and Amit Kumar Das. "Antidiarrheal and anti inflammatory activities of Lupeol, Quercetin, β - Sitosterol, Adene-5-en-3-ol and Caffeic acid isolated from Rhizophora Mucronata Bark."
18. Chi, Sang-Cheol, and H. Won Jun. "Anti-inflammatory activity of ketoprofen gel on carrageenan -induced paw edema in rats." Journal of pharmaceutical sciences 79.11 (1990): 974-977.
19. Lin, Chun-Ching, and Den-En Shieh. "The anti-inflammatory activity of Scutellaria rivularis extracts and its active components, baicalin, baicalein and wogonin." The American journal of Chinese medicine 24.01 (1996): 31-36.
20. Singh, Surender, D. K. Majumdar, and H. M. S. Rehan. "Evaluation of anti-inflammatory potential of fixed oil of< i> Ocimum sanctum</i>(Holybasil) and its possible mechanism of

action." Journal of ethnopharmacology 54.1 (1996): 19-26.

21. Hajare, S. W., et al. "Analgesic and antipyretic activities of Dalbergia sissoo leaves." Indian journal of pharmacology 32.6 (2000): 357-360.
22. Pathak, A. K., and A. Argal. "Analgesic activity of< i> Calotropis gigantea</i> flower." Fitoterapia 78.1 (2007): 40-42.
23. Malairajan, P., et al. "Analgesic activity of some Indian medicinal plants." Journal of ethnopharmacology 106.3 (2006): 425-428.
24. Screening methods in pharmacology by NS Parmar and Shiv Prakash on Laboratory animals Pg 37 and Evaluation of drugs acting Analgesic and anti-inflammatory Pg 211-238.
25. Screening methods in Pharmacology, volume-1 by Turner on Neuropharmacological tests in Blind screening Pg - 26, review on Analgesic Pg 100-117, Inflammation Pg 233-234.