***In Vivo* identification of pharmacological activities of *Malva neglecta* Wallr.**

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Abstract: The use of medicinal plants for cure, treatment and control of diseases is one of the ancient forms of medical practice in mankind history. The 80% population of underdeveloped nations is still dependent on traditional medicinal remedy for its basic medical needs. *Malva neglecta* Wallr. is a medicinal plant used for phytotherapy for the treatment of cough, respiratory and digestive problems. The present study was conducted for the identification of pharmacological activity of crude extract of *Malva neglecta* Wallr. on inflammatory, emesis, and analgesic effect in different *In-Vivo* models. For this study we performed hind paw, eddy hot plate, and check emesis methods. In the standard condition methods, 78.05%, 76.75% inhibition in anti-emetic, anti-inflammatory and in analgesic it shows significant effect. *Malva neglecta* Wallr. crude extract (Mn.Cr) possess anti-inflammatory, analgesic and antiemetic activities.

1 Introduction

The ethnopharmacology is defined as a scientific evaluation of biologically active substances traditionally used or observed by the man [1]. The definition of modern days interdisciplinary research involving plants, animals and the physiological role of other substances and the indigenous culture of drugs used in the past [2]. The drug development approach from plant resource depends on goal. The suitable choice of medicinal plant for study is very vital and essential step. There are number of conventional ways to accomplish this, including chemical composition, traditional use, toxicity and random selection. It is often desirable, possible and sometimes unavoidable to use combination of various criteria. Besides the chosen strategy, seeking database and scientific literature is critical in search for active compounds that have been identified and can also be used as a standard for selecting plants, if the purpose is to discover a novel source [3]. The use of medicinal plants for cure, treatment, and prevention of diseases is one of the ancient forms of the medical practice of humankind. The 80% population of underdeveloped nations still dependent on traditional medicinal remedy their basic medical needs [4]. Thousands of species of medicinal plants are utilized for preparation of herbal products with strong and effective drugs, established from centuries and could not be replaced by current allopathic preparations [5]. This traditional knowledge strongly needs to be preserved as 74% of all plants derived drugs are discovered through follow up investigation of ethnomedicinal knowledge [6].

Malva neglecta Wallr. (Mn) with synonyms of *Malva rotundifolia* from family of Malvaceae. *Malva neglecta* Wallr. is an herbaceous annual plant. The species *Malva* includes mucilage, malvine, tannin, and glucose. *M. neglecta* and some *Malva* species flowers and leaves are essential used for traditional phytotherapy [7]. They are used to treat cough, respiratory and digestive problem [8].

In this study different *In vivo* models (Rabbits, Chicks, and Albino Rats) were used for the investigation of the anti-piratic, anti-emetic, and anti-inflammatory effect of the ethanolic crude extract of *Malva neglecta* Wallr.

2 Materials and Methods

The plant was collected during April, and the plant was identified by an expert taxonomist, Assistant Prof. Dr. Zafar Ullah Zafar at the Institute of Pure and Applied Biology Baha Uddin Zakariya University Multan Pakistan and a voucher specimen was deposited in herbarium (F.PK-481). The plant material was shade dried. The material was grinded into powder by special herbal grinder. The 1 kg coarse powder was soaked in 70% aqueous ethanol in amber colored glass bottles at room temperature for 7 days with occasional shaking. The soaked material was passed through muslin cloth and the fluid obtained was filtered through Whatman-1 filter paper. The filtrate was stored in air tight container. The same procedure was repeated twice, and filtrates of all the three macerations were mixed. The filtrated was evaporated in a rotary evaporator under reduced pressure at 37°C to paste-like consistency with an approximate yield of 28%.

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2.1. Chemicals

Domperidone, copper sulfate pentahydrate, carrageenan, acetylsalicylic acid, sodium chloride was purchased from Merck Germany. The chemicals useful for these studies were almost of analytical rank.

2.2. Animals and housing conditions

The animals (Rabbits 1-2kg, Mice 27 – 20 g, Rats 170 - 190 g, Chicks 32-52) of either sex were purchased from local market for experimentation. These were maintained at Animal House of Faculty of Pharmacy, B.Z.U. Multan Pakistan under controlled conditions at temperature (25 °C - 27 °C) and provided with fresh green fodder and tap water ad libitum. The animals were subjected to overnight fasting before the experiments but were given free access to water. All experiments were carried out under conditions in accordance with the rulings of Institute of Laboratory Animal Resources, Commission on Life Sciences (NRC, 1996), approved by the Ethical Committee of B.Z.U., Multan Pakistan.

3 In vivo pharmacological study

3.1 Antiemetic activity by chick emesis model

The chicks of four days old and weighing from 32-52 g were purchased from market for antiemetic activity. The antiemetic activity was evaluated by using slight modified chick emesis model [9]. Each chick was placed in large beaker and allowed to stabilize for 10 minutes. The extract of *Malva neglecta* Wallr. was dissolved in 0.9% saline and administered at a dose of 150 mg/kg orally to the animals of test group (n=5). The saline 0.9% was received orally by negative control group (n=5). The Domperidone received by the positive control group (n=5) at a dose of 150 mg/kg orally as standard antiemetic drug. After 10 minutes the emesis agent copper sulphate pentahydrate was administered at a dose of 50 mg/kg then the number of retches was counted for 15 minutes. By using the following formula percentage of inhibition was calculated as follows:

$$\% \text{ Inhibition} = (A-B/A) \times 100$$

Where A are frequency of retching in control group and B are the frequency of retching in test group.

3.2 Effect of *Mn.Cr* on bleeding and clotting time of rabbits

The experiments were carried out on rabbits by giving cut on their ear lobe. The blood spots were taking on filter paper and in capillary tube. After taking the normal measuring the normal time of clotting and bleeding time the *Mn.Cr* was dissolved in normal saline and injected I.V at different concentrations of 1, 3 and 5 mg/kg to the rabbits. The bleeding and clotting time was recorded again after 30 minutes of injection. The procedure was repeated five times.

3.2.1 Anti-inflammatory activities by hind paw edema method

The albino rats (170 - 190 g) of either sex were used to determine the anti-inflammatory activity by using the standard method [10]. The animals were divided into 4 groups. Each group contain five animals. To induce the edema suspension of carrageenan 1% w/v in 0.9% saline was injected in sub-plantar right hind paw of the rats [11]. The test groups received ethanolic aqueous extract of plant orally at a dose of 300 and 500 mg/kg 1hr prior to the injection of carrageenan. The negative control group was given the normal saline of the same volume. The positive control group was treated orally 1hr before the injection of carrageenan with 300 mg/kg of acetylsalicylic acid. The inflammation of paw was determined by displacement of water in term of ml by edema in (Ugo Basil) playthysmometer before the injection of carrageenan and after 1, 2, 3, and 4 hours of carrageenan injection.

The percent inhibition of edema was calculated as follows:

$$\% \text{ inhibition} = (A-B/A) \times 100$$

3.2.2 Analgesic activity by hot plate method

The mice of either sex (27 – 20 g) were used and for evaluation of analgesic activity of test material by using the hot plate method performed by Eddy and Leimback [12]. The animals divided into three groups (n=5). Group one received 2 ml/kg normal saline. Group two was given plant extract at a dose of 100 mg/kg and third group was treated with acetylsalicylic acid 100 mg/kg intraperitoneally. One hour after administration of the plant extract the animals were sited on hot plate at 55 - 56°C. The time of paw licking or jump away taken by the animal was the time of reaction and measured at 0, 30 and 60 minutes.

4 Statistical Analysis

All data are presented as mean $\pm$ SD. Statistical analysis was done using GraphPad Prism 5.01 (GraphPad Software, Inc., La Jolla, CA, USA).

5 Results

5.1 Antiemetic effect of *Mn.Cr*

The mean number of retches in control was 64.10. Standard antiemetic drug domperidone showed 13.40 mean numbers of retches whereas *Malva neglecta* Wallr. extract showed 12.15 respectively. The percentage inhibition of emesis was noted as 78.05% whereas domperidone showed 76.78 % inhibition of emesis (Table 1 and Figure (a)).

Table 1: Anti emetic effect of *Mn.Cr* in chicks emesis model

Group	Mean Number of Retches $\pm$ S.E.M.	Inhibition (%) of Emesis
Control	64 $\pm$ 10	-----
Standard Domperidone 100mg/kg	13.4 $\pm$ 2.2	76.78%
<i>Mn.Cr</i> 150mg/kg	12.15 $\pm$ 1.14	78.05%

Fig (a): Comparison of anti-emetic activity of *Mn.Cr* against domperidone as an anti-emetic effect in chicks

Fig (b): Effect of *Mn.Cr* on bleeding time.

Fig (c): Effect of *Mn.Cr* on clotting time

5.2 Effect of *Mn.Cr* on bleeding and clotting time

The *Mn.Cr* increased the bleeding and clotting time of the rabbits at different concentrations 1, 3 and 5 mg/kg (Table 2 and Figure (b), (c))

Table 2: Effect of *Mn.Cr* on bleeding and clotting time of rabbits

Doses	Control Bleeding time (Sec)	Control clotting time (Sec)	Bleeding time after <i>Mn.Cr</i> injection (Sec)	Clotting time after <i>Mn.Cr</i> injection (Sec)
<i>Mn.Cr</i> 1mg/kg	2.45 $\pm$ 0.9	4.27 $\pm$ 1.56	3.60 $\pm$ 37	6.36 $\pm$ 37
<i>Mn.Cr</i> 3mg/kg	2.49 $\pm$ 2.47	4.09 $\pm$ 0.46	5.18 $\pm$ 64	6.26 $\pm$ 64
<i>Mn.Cr</i> 5mg/kg	2.45 $\pm$ 0.27	4.17 $\pm$ 0.1	8.38 $\pm$ 21	9.87 $\pm$ 1.37

5.3. Anti-inflammatory activity of *Mn.Cr*

Anti-inflammatory activity of the *Mn.Cr* extract was done against Carrageenan induced paw edema. The *Malva neglecta* Wallr. extract 300 mg/kg produced significant anti-inflammatory activity causing inhibition in edema to 37.54% at 4th hour. The extract of *Mn.Cr* reduced the edema induced by carrageenan at dose of 500 mg/kg by giving the paw volume of paw measured by pleythsmometer as 85% at 4th hour. The results of *Mn.Cr* at 500 mg/kg were found to be very significant. Whereas, the results of standard drug (ASA) in volume of paw measurement 76.75% at 4th hour (Table 3 and Figure (d)).

Fig (d): % inhibition of *Mn.Cr* at different time interval.

Table 3: Effect of two different concentration of *Malva neglecta* Wallr. on paw volume in carrageenan induced edema in rat in comparison to aspirin

Groups	Dose	Paw volume (ml)				
		0 h	1hr	2hr	3hr	4hr
saline	0.5 ml	0.298±0.053	0.598±0.053	0.642±0.038	0.687±0.043	0.718±0.061
Aspirin	300mg	0.226±0.031	0.508±0.031	0.529±0.029	0.440±0.014	0.428±0.014
Mn.Cr	300mg	0.227±0.054	0.592±0.054	0.596±0.042	0.556±0.029	0.522±0.030
Mn.Cr	500mg	0.288±0.044	0.568±0.044	0.546±0.048	0.519±0.027	0.468±0.010

5.4. Analgesic activity of *Mn.Cr*

Measurement of analgesic activity of *Malva neglecta* Wallr. The extracts of *Malva neglecta* Wallr when administered to mice, the time of reaction was recorded on minutes. The *Malva neglecta* Wallr. crude extract exhibited significant results. While the standard drug aspirin showed 15.10±1.19 and 19.32 ±1.93 after 30 and 60 minutes respectively (Table 4 and Figure (e)). hot plate. The *Malva neglecta* Wallr. extract showed time of reaction 15.10±1.19 and 19.32 ±1.93 after 30 and 60

Table 4: Analgesic effect of *Malva neglecta* Wallr. on mice in comparison to aspirin

Groups	Dose (mg/K g)	Licking of Paw (normal)	After drug administration(min)		
			0	30	60
Control	-----	4.8±0.8	4.25±0.9	5.22±0.95	4.34±1.76
Aspirin	100	4.39±1.39	15.10±1.19	19.32 ±1.93	22.28±0.10
Mn.Cr	100	4.19±0.29	7.19±1.28	11.17±1.72	13.75±0.083

Fig (e): Time of licking taken by mice after administration of crude extract *Malva neglecta* Wallr. & aspirin at different time intervals

6 Discussion

The *Mn.Cr* increased the bleeding and clotting time in rabbits. These effects may be due to inhibition of the thromboxane A₂ which increases the platelet aggregation [13]. The bleeding time and platelet aggregation is inversely related to each other [14]. Anti-inflammatory activity of *Mn.Cr* was also evaluated. The crude extract of *Mn.Cr* inhibited edema and more pronounced effect was seen in the 4th hour NSAIDs like activity. The standard

drug acetylsalicylic acid (ASA) was used. ASA caused reduction in inflammation as previous studies showed anti-inflammatory effect of NSAIDs on rat induced by Carrageenin-injection [15]. Carrageenin-induced rat paw edema basically reveals the action of prostaglandin's which is concerned with the mechanism of inflammation [16]. Edema formation on rat paw is due various inflammatory mediator that increases vascular permeability [17]. Edema induced by carrageenin is biphasic. In first phase it produces the chemical mediator such as histamine, bradykinin lasting for 45 to 60 minutes. In 2nd phase prostaglandins react in the development of the inflammation [18]. It has been reported that non-steroid anti-inflammatory drugs (acetylsalicylic acid) decrease the pain sensation and inflammation by inhibiting the prostaglandin synthesis [19].

Analgesic activity of *Mn.Cr* was also screened by Eddy's hot plate method. Hindpaw licking has been worthy reference point of nociception in hot plate test [20]. The hot plate method is well reliable for centrally acting drugs [21]. Any material that caused the prolongation latency time to hot plate must be an agent acting centrally [22]. The animals treated with plant extract and acetylsalicylic acid (100 mg/kg at 90 minutes) spent longer time without licking of paw than the animals of control group on hot plate. The group of animals treated with *Mn.Cr* showed longer latency to hot plate in first 30 minutes than other control and standard group. The *Mn.Cr* revealed both types of pain inhibition, therefore it must have a peripheral and central activity.

Analgesic effect of plant extract may be due to central mechanism involving receptor system (dopaminergic, opiate or serotonergic systems) or may be due peripheral mechanism such inhibition of prostaglandins, leukotrienes or other chemical mediator [23].

In the present study the ethanolic extract of the *Malva neglecta* Wallr was investigated for antiemetic effect. The percentage of inhibition of retches was noted as 78.05% by *Mn.Cr* whereas domperidone showed 76.78% inhibition in retches. The *Mn.Cr* showed antiemetic effect comparable with domperidone. The *Mn.Cr* may have a peripheral antiemetic action as the copper sulphat administered orally induced emesis by peripheral action [24] and plant extract was more protective against its action.

7 Conclusion

The anti-inflammatory activity of *Mn.Cr* has been evaluated by the authors. It has been found out that the extract of *Mn.Cr* inhibited edema and the effect was comparable with the NSAID acetylsalicylic acid. Additionally, analgesic activity was also evaluated by using the Eddy's hot plate method. The analgesic effect of plant extract envisaged on the basis of the central mechanism involving receptor system (dopaminergic, opiate or serotonergic systems) or may be because of peripheral mechanism such inhibition of prostaglandins, leukotrienes or other chemical mediator. It is pertaining to mention that the crude extract of *Mn.Cr* also have the antiemetic activities, which is comparable with the drug domperidone. Author expect that present study act as a

milestone in the natural product utilization in the common health issues.

References

1. B Holmstedt, J Ethnopharmacol **32**, 7 (1991)
2. M.Giorgetti, G. Negri, E. Rodrigues, J Ethnopharmacol **109**, 335 (2007)
3. M.S. Rates, *Toxicol* **39**, 603 (2001)
4. A.Mahmood, A.Mahmood, R.N.Malik, Z.K Shinwari, *J Ethnopharmacol*, **148**, 714 (2013)
5. I.Ahmad, Z.Mehmood, F.Mohammad, *J Ethnopharmacol* **62**, 183 (1998)
6. A. B. Gulshan, A. A. Dasti, S. Hussain, M. I. Atta, *J med plants* **7**, 750 (2012)
7. A. Güder, H. Korkmaz, *IJPR* **11**, 913 (2012)
8. Baytop, T J, *istanbul Univ publ*, **418**, (1984)
9. Y. Akita, Y. Yang, T. Kawai, K. Kinoshita, K. Koyama, K. Takahashi, Watanabe, *Nat Prod Sci*, **4**, 72 (1998)
10. Winter, A. Charles, *J Am Pharm Assoc Am Pharm Assoc* **46**, 515 (1957)
11. M.Ocete, S. Risco, A. Zarzuelo, J. Jimenez, *J Ethnopharmacol*, **25**, 305 (1989)
12. N B. Eddy, D. Leimbach, *J Pharmacol Exp Ther* **107**, 385 (1953)
13. D. Payne, P. Hayes, C. Jones, P. Belham, A. Naylor, *J. Vas. Surg* **35**, 1204 (2002)
14. L. A. Harker, S. J. Slichter, *N Engl J Med* **287**, 155 (1972)
15. Y. OZAKI, L. XING, L.; M. SATAKE, *Biol Pharm Bull* **19**, 1046 (1996)
16. A. Mujumdar, A.V.Misar, *J Ethnopharmacol*, **90**, 11 (2004)
17. A. Ialenti, A. Ianaro, S. Moncada, M. D. Rosa, *Eur J Pharmacol* **211**, 177 (1992)
18. S.H. Ferreira, *Br. J. Clin. Pharmacol* **10**, 237S (1980)
19. J. R. Vane, *Nat New Biol* **231**, 232 (1971)
20. O.G Berge, O. B. Fasmer, K. Hole, *Pharmacol Biochem. Behav* **19**, 873 (1983)
21. E. Sabina, S. Chandel, M. Rasool, K. Mahaboob, *Int J Integr Biol* **6**, 52 (2009)
22. G.F. Ibironke, K.I. Ajiboye, *IJP* **3**, 177 (2007)
23. S. Suseem, M. Saral, N. Reddy, M. Gregory, *Asian Pac. J. Trop. Med* **4**, 21 (2011)
24. H. Hossein, M. Mashallah, G. Irn *J Pharm Sci*, **1**, (2005)