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ANTI-INFLAMMATORY ACTIVITY OF SOME MEDICINAL PLANT EXTRACTS ON ALBINO MICE

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Abstract: Herbal therapy is well recognized across world and in many cultures. Even till 21 Century it accounts as an unwritten science in rural world to treat many diseases. Like other chronic conditions, inflammatory diseases are standing first in a row getting global attention. Inflammation includes diapedesis resulting in migration of the white blood cells in inflamed part and protecting body from infection with foreign organisms like bacteria and viruses. Several reports over crude plant extracts have shown to be effective in such cases. The action of poly-herbs differs in many respects from the actions of single pure drugs or synthetic drugs. The activity of these crude drugs depends on several factors like method of administration, potentiality and safety. Since methanol is the most common and efficient solvent as compared to chloroform and petroleum ether for preparing drug formulations. By the other hand leafs of Aloe vera L., Lawsonia inermis L. and Vitex negundo L. are well known for their biological activities. During present investigation 57.90 percent anti-inflammatory activity was seen with crude extract of Aloe vera L., similarly Lawsonia inermis L. alone showed 51.75 percent activity, whereas Vitex negundo L. showed highest topical anti-inflammatory activity i.e. 58.78 percent. All crude drugs showed activity at 200 mg/kg per body weight of albino mice. Modified mercury displacement method proves to be very effective for the evaluation of anti-inflammatory activity using mice paw edema test.

Keywords: *Anti-inflammatory activity, Albino mice, Aloe vera L., Lawsonia inermis L., Mercury displacement, Paw edema Vitex negundo L.*

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I INTRODUCTION

Herbal therapy is well recognized across world and in many cultures. Even till 21 Century it accounts as an unwritten science in rural world to treat many diseases. Ayurveda or traditional Indian medicines are becoming increasingly popular across globe as many chronic conditions like Asthma, Arthritis, Autoimmunity, Cancer, Heart Disease, Neuro-degeneration, Obesity are well being treated without any side effects. Like other chronic conditions, inflammatory diseases are standing first in a row getting global attention. Inflammation includes diapedesis resulting in migration of the white blood cells in inflamed part and protecting body from infection with foreign organisms like bacteria and viruses. Therefore the inflammation is a vital part of the immune system's response to injury. Although this, if the inflammatory response withstands for longer time it may cause problems in vital organs. For example chronic inflammation is found to be linked with hearts disease or stroke, and also causes autoimmune disorders like rheumatoid arthritis. Several reports over crude plant extracts have shown to be effective in such cases. The action of poly-herbs differs in many respects from the actions of single pure drugs or synthetic drugs. The activity of these crude drugs depends on several factors like method of administration, potentiality and safety. Since methanol is the most common and efficient solvent as compared to chloroform and petroleum ether for preparing drug formulations. By the other hand leafs of *Aloe vera* L., *Lawsonia inermis* L. and *Vitex negundo* L. are well known for their biological activities. Therefore present study was aimed to investigate topical anti-inflammatory activity on albino mice, as insufficient literatures and fewer studies are available regarding their topical activity.

II MATERIALS AND METHODS

For study all analytical reagents, borosilicate glass wares were made available from biotechnology research centre at D.B.F Dayanand College of arts and science, Solapur

In present research investigation anti-inflammatory activity of crude methanol extracts of different screened medicinal plants was checked using albino mice [11].

Collection and identification of medicinal plants

All plant materials were collected from western parts of Maharashtra state, India. Reproductive parts like flower or solid parts were skilfully preserved for the identification. Identification was by a botanist at D.B.F Dayanand College of arts and science, Solapur.

Extraction of medicinal plant parts

Shade dried and powdered plant materials were powdered using electric grinder. Later powdered plant material was extracted using pure cold methanol for successive 7 days (1 kg/litter), as per methodology described by Dama *et al.* [6]. The contented extracts were cleaned through Whatmann's filter paper. Extraction was done using methodology agreed by Dieu *et al.* [8].

In vitro screening of medicinal plants for anti-inflammatory activity

All medicinal plants were screened using protein digestion assay as described by Banerji [1,4,5,7]. All 3 plants were screened at concentration from 50 to 200 mg /kg for anti-inflammatory activity as shown in table 2.

Partial purification of crude extracts using Colum chromatography

All extracted and screened medicinal plant extracts were partially purified by using column chromatography, methodology described by Dama *et al.* [6]. Using Hexane - Acetone eluents, five fractions were obtained. From these, fraction E shows high bio-active metabolites in it [9, 10]. Hence fraction E was chosen for further animal studies.

Animal collection and maintenance of albino mice

For the present research work 18 albino mice of either sexes were obtained from 'Aarya Biotech Pvt. Ltd, Dhule (M.S), India and housed as per CPCSEA guidelines laid down by (CPCSEA, 2003). All animals were feed with adequate supplies of food and fresh water.

Anti-inflammatory activity on albino mice

Anti-inflammatory activity of selected medicinal plant extracts was checked topically (externally) on albino mice paw. Inflammation in mice left paw was induced using 0.1 ml of 1 % carrageenan suspension by direct injection [6, 9]. For the testing, all animal were dispersed in four groups (Group I- IV) pertaining six animals in each group as shown in table 1.

Table 1. Groups of animal used for anti-inflammatory activity

Group I	0.1 ml of 1% carrageenan (Control Group)
Group II	0.1 ml of 1% carrageenan with propellant (Negative control)
Group III	Diclofenac 10 mg/kg (Positive control)
Group IV	Test group 200 mg/kg (Experimental group)

Mercury displacement technique for activity assessment

Mercury displacement technique was used present research work to estimate extent of swelling. Mice swollen paw was kept poignant the wall of the calibrated column containing the mercury fluid. Every half hourly displacement in mercury was noted and corresponding readings displayed were receded ensuring less error [6, 13, and 16].

Percentage inhibition calculation

A decrease in paw inflammation was articulated in percent inhibition using formula given by Elsharkawy *et al.* [9].

$$\frac{(\text{Final volume (Vt)} - \text{Initial volume (Vo)})}{\text{Initial volume (Vo)}} x100$$

Where 'Vt' is final paw volume displacement and 'Vo' is the initial paw volume displacement.

Statistical analysis

Noting's from screening and anti-inflammatory activity were amassed by using standard statistical methods. The experimentation was performed on mice groups in order to confirm the reproducibility of the result. All values from mercury displacement were expressed as Mean ± S.E.M. One way analysis of variances (one way ANOVA) was used for calculating

statistical significance at $p<0.05$ methodology described by Dieu *et al*. [8].

III RESULTS

Collection and identification of medicinal plants

For present research work medicinal plants were collected from western parts of Maharashtra, India. All collected plant materials were identified by botanist from the Department of Botany, and the Herbarium sheaths, were deposited in the Department of Zoology, D.B.F Dayanand College of Arts and Science Solapur. Thereafter all plant materials were macerated in electric blenders for getting fine powder for extraction.

Extraction of medicinal plant parts

Extraction of plant materials was done was per methodology discussed by Dama *et al.* [6]. During consecutive seven days of abstraction from 1 kg of powdered sample 20 gm sticky material was recovered. Collected material was stored in dry place procedure given by Dieu *et al*. [8].

***In vitro* screening of medicinal plants for anti-inflammatory activity**

All crude methanol extracts of selected medicinal plant extracts were found to be very active at concentration of 200 mg/kg of protein weight. Method described by described by Banerji *et al*. (2014) proves to be very effective.

Table 2. Screening of medicinal plants for their anti-inflammatory activity

Sr. No.	Scientific name	Parts (used)	50mg /kg	100mg /kg	150mg /kg	200mg /kg
1	*Aloe vera* L.	Leaf	+	+	+	+++
2	*Lawsonia inermis* L.	Leaf	+	+	+	+++
3	*Vitex negundo* L.	Leaf	+	+	+	+++

Partial purification of crude extracts using Colum chromatography

All extracted and screened medicinal plant extracts were partially purified by using column chromatography, methodology described by Dama *et al.* [6]. Using Hexane - Acetone eluents, five fractions were obtained. From these, fraction E shows high bio-active metabolites in it [6, 8, 9, and 10]. For present work fraction E was chosen for further animal studies.

Animal collection and maintenance of albino mice

For the present research work 18 albino mice of either sex were obtained from Aarya Biotech Dhule (M.S), India (CPCSEA registration No. 1822/PO/RcBiBt/S/15 /CPCSEA). All animals were housed as per CPCSEA guidelines laid down by (CPCSEA, 2003). All animals were feed with adequate supplies of food and fresh water.

Inflammatory activity of 1 % carrageenan and propellant on albino mice paw

0.1 ml carrageenan in sub planter left paw albino mice induced 100 % inflammation in 4 hours of administration. However there was no natural inflammation reduction seen even after 4 hours [1,2]. Therefore carrageenan was used as positive control during study (group I). Figure 4 also shows that propellant alone had no anti-inflammatory activity on paw (group II). Hence it was taken as negative control (group II). In all cases graph was plotted with time in hour against mercury displacement in ml [6, 9, and 21].

Anti-inflammatory activity on albino mice

The topical anti-inflammatory activity of *Aloe vera* L., *Lawsonia inermis* L., *Vitex negundo* L. leaf extracts, was evaluated with modified mercury displacement method technique described by Poul *et al*. [10], in 0-4-hour time intervals. Results were compared with standard 10 % diclofenac spray. All readings were expressed as Mercury displacement (ml)/ Hour, with average mean ± S.E.M [7, 9].

***Aloe vera* L.**

During defined time intervals *Aloe vera* L. leaf extracts showed notable activity throughout 2-4 hours. Maximum activity was realized 2 to 2 ½ hours after topical application of 200 mg/kg extract. In table mean 0.126 ± 0.0014 shows zero percent inflammation (normal paw) whereas mean value 0.304 ± 0.0022 indicates 100 % inflammation in initial half hour (inflamed paw). There was significant reduction inflammation seen every half hour. Overall diclofenac spray showed 68.89 percent activity, whereas *Aloe vera* L. crude extract showed 58.59 % inflammation reductions in 4 hour, which can be read from 0.197 ± 0.0026 values. Results summarized in table 7 analyses comparative activity between *A. marmelos* L. and standard diclofenac drug. One way ANOVA f - values 5.3 were found to be significant at $p< 0.05$ as shown in table 3

Table 3. Anti-inflammatory activity of *Aloe vera* leaf L. extract on albino mice left paw (Mean ± S.E.M).

Animal Group	Mercury displacement (ml)/Hour (Average volume Mean ± S.E.M)									Activity (%)
	Initial hour	½ hour	1 hour	1 ½ hour	2 hour	2 ½ hour	3 hour	3 ½ hour	4 hour	
Group I	0.130 ± 0.0037	0.302 ± 0.0022	0.303 ± 0.0017	0.299 ± 0.0017	0.294 ± 0.0022	0.292 ± 0.0023	0.292 ± 0.0022	0.289 ± 0.0023	0.289 ± 0.0031	0
Group II	0.131 ± 0.0037	0.304 ± 0.0022	0.304 ± 0.0022	0.299 ± 0.0017	0.289 ± 0.0017	0.290 ± 0.0026	0.287 ± 0.0034	0.282 ± 0.0048	0.287 ± 0.0022	0
Group III	0.127 ± 0.0035	0.300 ± 0.0017	0.277 ± 0.0035	0.277 ± 0.0035	0.258 ± 0.0048	0.243 ± 0.0048	0.210 ± 0.0048	0.195 ± 0.0048	0.177 ± 0.0035	68.89
Group IV	0.126 ± 0.0015	0.304 ± 0.0023	0.304 ± 0.0023	0.297 ± 0.0030	0.259 ± 0.0025	0.272 ± 0.0020	0.241 ± 0.0024	0.212 ± 0.0020	0.197 ± 0.0026	57.90

***Lawsonia inermis* L.**

Table 4. Anti-inflammatory activity of *Lawsonia inermis* L. leaf extract on albino mice left (Mean ± S.E.M).

Animal Group	Mercury displacement (ml)/Hour (Average volume Mean ± S.E.M)									Activity (%)
	Initial hour	½ hour	1 hour	1 ½ hour	2 hour	2 ½ hour	3 hour	3 ½ hour	4 hour	
Group I	0.130 ± 0.0037	0.302 ± 0.0022	0.303 ± 0.0017	0.299 ± 0.0017	0.294 ± 0.0022	0.292 ± 0.0023	0.292 ± 0.0022	0.289 ± 0.0023	0.289 ± 0.0031	0
Group II	0.131 ± 0.0037	0.304 ± 0.0022	0.304 ± 0.0022	0.299 ± 0.0017	0.289 ± 0.0017	0.290 ± 0.0026	0.287 ± 0.0034	0.282 ± 0.0048	0.287 ± 0.0022	0
Group III	0.127 ± 0.0035	0.300 ± 0.0017	0.277 ± 0.0035	0.277 ± 0.0035	0.258 ± 0.0048	0.243 ± 0.0048	0.210 ± 0.0048	0.195 ± 0.0048	0.177 ± 0.0035	68.89
Group IV	0.126 ± 0.0042	0.304 ± 0.00211	0.277 ± 0.0042	0.276 ± 0.0034	0.259 ± 0.0073	0.246 ± 0.0412	0.206 ± 0.0412	0.226 ± 0.0096	0.203 ± 0.0168	51.75

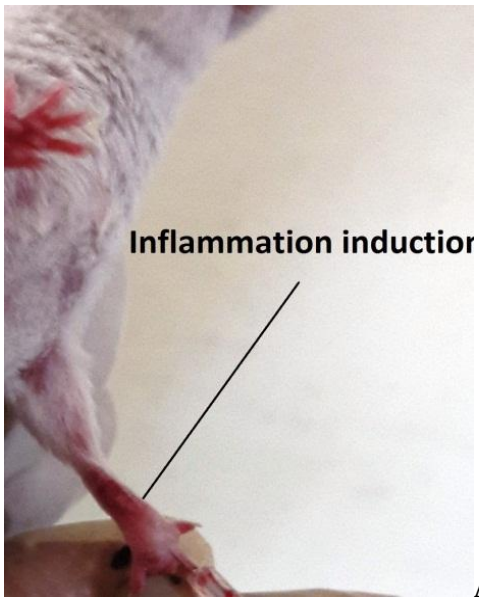
Table 4 shows anti-inflammatory activity of *Lawsonia inermis* L. The crude extract showed 51.75 % anti-inflammatory activity with 0.203 ± 0.0168 mercury displacement in 4 hours as shown in table 4.

***Vitex negundo* L.**

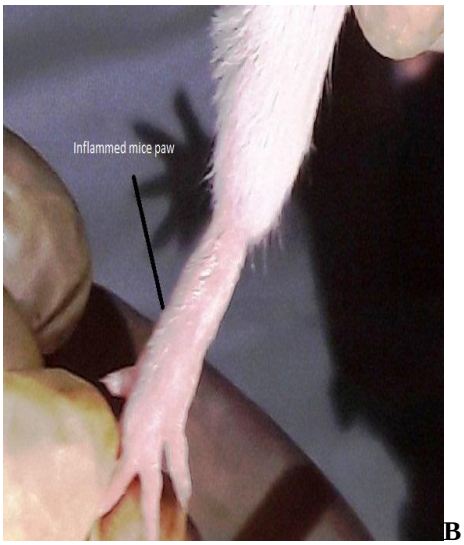
Table 5. Anti-inflammatory activity of *Vitex negundo* L. leaf extract on albino mice left paw (Mean ± S.E.M).

Animal Group	Mercury displacement (ml)/Hour (Average volume Mean ± S.E.M)									Activity (%)
	Initial hour	½ hour	1 hour	1 ½ hour	2 hour	2 ½ hour	3 hour	3 ½ hour	4 hour	
Group I	0.130 ± 0.0037	0.302 ± 0.0022	0.303 ± 0.0017	0.299 ± 0.0017	0.294 ± 0.0022	0.292 ± 0.0023	0.292 ± 0.0022	0.289 ± 0.0023	0.289 ± 0.0031	0
Group II	0.131 ± 0.0037	0.304 ± 0.0022	0.304 ± 0.0022	0.299 ± 0.0017	0.289 ± 0.0017	0.290 ± 0.0026	0.287 ± 0.0034	0.282 ± 0.0048	0.287 ± 0.0022	0
Group III	0.127 ± 0.0035	0.300 ± 0.0017	0.277 ± 0.0035	0.277 ± 0.0035	0.258 ± 0.0048	0.243 ± 0.0048	0.210 ± 0.0048	0.195 ± 0.0048	0.177 ± 0.0035	68.89
Group IV	0.127 ± 0.0042	0.297 ± 0.0021	0.297 ± 0.0021	0.287 ± 0.0042	0.266 ± 0.0072	0.249 ± 0.0060	0.229 ± 0.0075	0.207 ± 0.0055	0.191 ± 0.0044	58.78

Vitex negundo L. crude leaf extract showed determined activity among all screened plants. Upstretched inflammation in group IV was seen after half hours with 0.297±0.0042 of mercury displacement. Total 58.78 % inflammation reduction was noted in 4 hours of which can be interpreted from 0.190 ± 0.0044 mercury displacements (Table 5)



A



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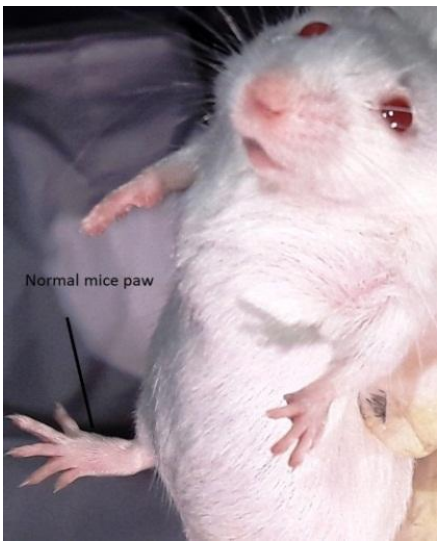

C

D

Figure 1. Anti-inflammatory activities of albino mice (A). 1% Carrageenan administration (B). Inflamed mice paw (C). Normal paws (D). Topical application of crude drug on inflamed paw

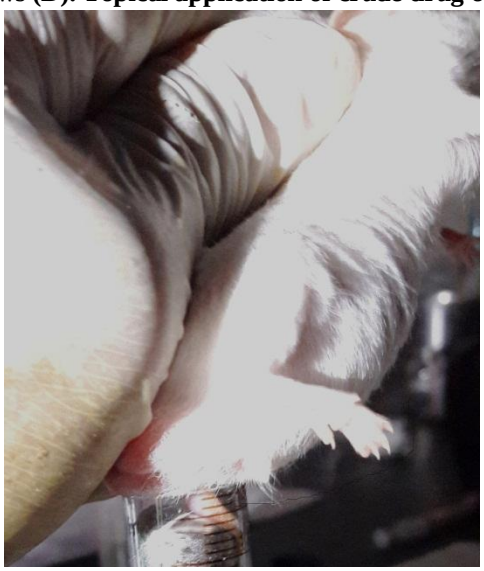

Figure 2. Mercury displacement technique for activity assessment

IV DISCUSSION

Serious health concerns due to inflammations have led us to find novel anti-inflammatory drugs using novel medicinal plants. Chakraborty *et al* [4], discussed the process of inflammation in reference with production of chemotactic compounds like prostaglandin, adhesive protein receptor action, interleukin or other chemo toxin [14]. These particles generate stress on the skin and thus activate the stimulation of hydrolysis of phospholipid by phospholipase A into arachidonic acid, which are membrane bound. Currently available synthetic drugs inhibit these chemo toxic molecules. These commonly available steroidal drugs are well known to provoke serious health problems and may lead to allergic responses (Xu *et al*., 2019). On contrary medicinal plants do not show any side effects which are in concerns with vital organs. A number study so far have been reported for the anti-inflammatory activity using medicinal plants around the globe for example, America [17, 18], India [10,13,14,15].This research investigation underwrites information in the database of Indian and world ethano-pharmacy.

Anti-inflammatory activity on albino mice

Present research investigation represents topical anti-inflammatory activity of selected medicinal plants extracts on mice paw [14,15,17] . Hypothetically screened plants included for animal experimentation exhibited substantial anti-inflammatory activity at 200 mg/kg per body weight of albino mice. These results are also analogous results discussed by Prabhu *et al*. [11]; Rajaram *et al.* [12]; Venkata and Rao, [16]; Dangar and Patel, [7]. In present research work 1 %, 0.1ml carrageenan shows effective for inflammation (100 %) induction in 0-4 hours in mice paw, as also formerly described by Susanna *et al.* [16,17]. During present investigation a great variation in pharmaco-kinetic anti-inflammatory activity was observed in selected plants extracts. Leaf extract *Aloe vera* L showed 57.90 % activity similar kind of results were also discussed by Bhalsinge *et al*. [3]. Similarly leaf extract of *Lawsonia innermis* L. showed 51.75 % activity, these results are comparable with results discussed by Barupal *et al.* [2]. Among all maximum anti-inflammatory activity was noted with leaf extract of *Vitex negundo* L. 58.78 % [19, 21].

V CONCLUSION

From obtained observations and results, it may be concluded that leaf extracts of *Aloe, Lawsonia and Vitex* at 200mg/kg per mice body weight shows high topical anti-inflammatory activity in spray formulation. Pure methanolic extract yield was up to 20 gm/kg. Very less work has been done with same approach. Further standardization in spray formulation and assessment method may improve quality in results. The same procedure may be applied with different crude plant extracts.

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