IJPSR (2020), Volume 11, Issue 10 (Research Article)

INTERNATIONAL JOURNAL OF PHARMACEUTICAL SCIENCES AND RESEARCH

Received on 24 October 2019; received in revised form, 24 March 2020; accepted, 26 March 2020; published 01 October 2020

AN EXPERIMENTAL STUDY TO EVALUATE ANTI-INFLAMMATORY ACTIVITY OF MURIVENNA IN WISTAR ALBINO RATS

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Keywords:

Murivenna, Acute Inflammatory Study, Chronic Inflammatory study, Plethysmography technique, Cotton pellet granuloma pouch method

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ABSTRACT: *Murivenna* is a *taila* (oil) which is widely used in South-Indian practices for external application in many of the localized conditions such as trauma, inflammation of joint, fracture, joint dislocation, wound, *etc*. *Taila* (Oil) is considered best for *vatahara* action. *Vata* is the main *dosha* involved in *shopha* (inflammation)*;* this is a suitable *taila* (oil) that works in the treatment of *shopha* (inflammation). **Materials and Methods:** Murivenna was prepared as per the guidelines of Trivandrum Pharmacopeia and Ayurveda Formulary of India with genuine drugs. A later experimental study was conducted in acute and chronic inflammation using Carrageenan-induced paw edema and cotton pellet granuloma pouch method in acute and chronic inflammatory study, respectively. The data were analyzed statistically using a one-way analysis of variance (ANOVA) followed by multiple comparison Dunnett's test as a post hoc test. **Result:** The results indicate the presence of significant anti-inflammatory activity in the experimental model representing acute inflammation and non-significant effect in chronic inflammation. But there is a decrease in the granuloma formation, which suggests that *Murivenna* can be given in granuloma condition. **Conclusion:** *Murivenna* retains anti-inflammatory activity in acute inflammation than in chronic.

INTRODUCTION: Inflammation is a local defense response. The cardinal signs are - Rubor (redness), Tumor (Swelling), Calor (Heat), Dolor (Pain), function laesa (loss of function). It is of 2 types - acute inflammation and chronic inflammation. Sprain, physical injury, suppression of marmas, or going against the dietic regimen may result in the disturbances of physical phenomenon and thus induce *Shopha*. *Muṛivenna* is a *taila* (oil) which is used for external application in many of the localized condition such as trauma, inflammation of joint, fracture, joint dislocation, wound, *etc*.

QUICK RESPONSE CODE

DOI: 10.13040/IJPSR.0975-8232.11(10).5091-03

This article can be accessed online on www.ijpsr.com

DOI link: http://dx.doi.org/10.13040/IJPSR.0975-8232.11(10).5091-03

Here an attempt is made to understand an externally used medicine known as *Murivenna* for its *shophahara* property (Anti-inflammatory action).

Aims and Objectives: To evaluate the Anti-inflammatory property of *Murivenna* in Wistar albino rats

MATERIALS AND METHODS:

Preparation (5/3/2018 to 10/3/2018): The ingredients are *Narikela (Cocos nucifera), Tambula* (*Piper betle*), *Sigru (Moringa oleifera), Paribhadra (Erythrina indica), Kumari (Aloe barbadensis), Karanja (Pongamia pinnata), Buka (Spermacocea hispida), Palandu (Allium cepa), Tambula (Oryza sativa), Satavari (Asparagus racemosus)*. These were freshly collected and authenticated. *Murivenna* was prepared as per Ayurveda Formulary of India [1] / Trivandrum Ayurveda Pharmacopeia [2] in the Lab attached to the

Department of Rasashastra and Bhaishajya Kalpana, Amrita School Of Ayurveda. Oil was prepared in khara paka (3rd stage of processing) and was later filtered and stored in a container. It took 3 days for the completion of preparation.

Experimental Study -7/5/2018 to 2/7/2018:
Test Drug Used:
Murivenna: Prepared and was stored in clean air tight container.

Standard Drug Used:
Diclofenac Sodium Gel:
Brand Name: Voveran Emulgel

Diclofenac belongs to the non-steroidal anti-inflammatory drug (NSAID), which works on pain and inflammation in the body. Diclofenac is used to treat mild to moderate pain and signs and symptoms of osteoarthritis, rheumatoid arthritis *etc*.

Selection of Animals: Healthy Wistar albino rats, which weighs **Fig. 1** in the range of 140 - 330 g were taken from the animal house of S.D.M. Centre for Research in *Ayurveda* and Allied Sciences, Udupi. The animals were housed in standard laboratory condition by exposure to natural day and night cycle with temperature of 25 ºC and 50-70% relative humidity in well-ventilated conditions kept in polypropylene cages. Animals were provided with normal mouse chow (Sai Durga Food and Feeds or Scientist Choice Laboratory Animal Feed, Bangalore, India) and water ad libitum **Fig. 3**.

The animals were randomly selected, marked with picric acid to permit individual identification, and kept in respective cages **Fig. 2** for 7 days prior to the start of the application of medicine to allow for acclimatization to the laboratory conditions. The experimental study was conducted after obtaining permission from the institutional ethics committee in accordance with the guideline formulated by CPCSEA. Approval No: SDMCRA/IAEC/AM-R-25,IAEC 29- 05-2017

Acute Inflammatory Study: The effect of test drug on carrageenan-induced paw edema in rats was studied as described by Winter, *et al*., 1962. Pregnant female rats were excluded. Healthy Wistar albino rats of either sex were weighed between 140-330 g, and it is randomly divided into four groups, **Table 1,** each consisting of 6 rats.

TABLE 1: GROUPING OF ANIMALS IN ACUTE INFLAMMATORY STUDY

Group I	Water control (Control Group)
Group II	Castor Oil (Castor Control group)
Group III	Diclofenac Sodium Gel (Standard group)
Group IV	*Murivenna* (Trial Group)

Total 24 rats, 6 each in the separate cage was maintained separately at the institute's animal house and were exposed to natural day and night cycles. Only tap water and food were administered to the first group so as to serve as a control. The second group, third group, and the fourth group was treated externally with castor oil, diclofenac sodium gel, and Murivenna, respectively. The drug was applied once daily for 3 consecutive days, and on the 4th day, the main procedure is done using the plethysmography technique where basal paw volume reading, 1st hour, 3rd hour, and 6th hour reading was taken, and again the drug is applied on the 5th day and 24th-hour reading was taken.

Steps Involved while using Digital Plethysmometer (Fig. 7) Filling of Water Cell: Plethysmometer consists of 2 glass stoppers where one is used for a water bath with the soap solution and the other one is used for dipping the rat paw in the soap solution drained from water bath for getting the reading. Here soap solution is used for conductivity. The water bath is filled with the soap solution and distilled water till the conductivity meter shows 240 µL and later, it is drained to the water cell, where 1ml of water is removed using a syringe after the filling of the cell.

Turning on the Unit: Main power was switched on. Every time when the unit is turned on, the unit will program by itself. The number will be displayed, which will be changed after a few seconds automatically to zero. To zero the display after each reading, press the reset button so that the reading will display as 0.0 or 0.00.

Calibration: Calibration was done by dipping 0.5ml, 1ml, and 2ml nobe. If we get the correct reading on the dipping of the nobe, the instrument is said to be calibrated and can be used for further reading of rat paw.

Reading: The reading will show the volume displaced when the paw is dipped into the soap solution. Zero may vary between 0 and 0.1 or 0 and 0.02 max. The minor deviation has to be tolerated.

The reading of rat paw is noted when it stops blinking or changing for a few seconds in the plethysmometer. Later again reset button is pressed to return to the normal reading, where the paw is removed from the glass stopper.

Experimental Procedure for Acute Inflammation: To the first group, tap water was administered to serve as a control. Second, third and fourth groups were treated with castor oil, diclofenac sodium gel, and Murivenna, respectively. The test drug was applied twice daily for three consecutive days, whereas the 2nd and 3rd group was applied only once a day for three consecutive days before the main procedure.

The study started with the trial group on 7/5/2018, where the application of Murivenna was done twice daily (morning 9.00 am and evening 3.30 pm) for 3 consecutive days till 9/5/2018 **Fig. 4**. 250 ml bottle was filled with tap water and was administered to the rats to ensure uniform hydration. This was supposed to minimize the variation in edema formation during the course of study. On 10/5/2018 the main procedure was conducted using a plethysmographic technique where the procedure started after calibration of the instrument, and then the basal paw volume was noted. Later by 10.00am paw oedema was induced by injecting **Fig. 5** 0.1 ml freshly prepared 1% Carrageenan in distil water *i.e.*, 50mg carrageenan in 5 ml distil water was prepared and 0.1 ml was then subjected to the sub-plantar aponeurosis of the left hind paw **Fig. 6**. Oil was applied every one hour and reading was noted 1st hour, 3rd hour, 6th hour and 24th hour **Fig. 8**.

This procedure was also done for castor control, standard group, and control group. The result was expressed as a percentage increase in paw volume in comparison to the initial value. The percentage increase in paw volumes was calculated by subtracting the initial paw volumes from the paw volumes obtained after the injection of the phlogistic agent. The figure was divided by initial paw volume and then multiplied by a hundred.

% Change = (Hour reading – Basal reading) × 100 / Basal reading

Statistical analysis was done to evaluate the efficacy of Murivenna in comparison to castor control and standard group.

Chronic Inflammation:

TABLE 2: GROUPING OF ANIMALS IN CHRONIC INFLAMMATORY STUDY

Group I	Water control (Control Group)
Group II	Diclofenac Sodium Gel (Standard group)
Group III	*Murivenna* (Trial Group)

The rats were anesthetized with ether **Fig. 11**. The Dorsum of neck was shaved **Fig. 10** and swabbed with 70% (v/v) alcohol. In the intracapsular region, midline incision of 1cm was made. A small tunnel was made on either side of the incision with small blunt forceps. One sterile cotton pellet **Fig. 9** weighing 100 mg (prepared by cutting rolled cotton into pieces of 100 mg and sterilized by autoclaving for 30 min under 15 lbs pressure) was inserted into the tunnel on either side of the incision **Fig. 12** and the incision was closed with interrupted sutures **Fig. 13** after expelling the air from the tunnel.

Group, I was treated with tap water and taken as a control group. Group II and III **Table 2** was taken as standard and trial group which is treated with Diclofenac sodium gel and *Murivenna* **Fig. 14,** respectively, for 7 consecutive days starting from the day of implantation. The rats were sacrificed on 8th day after taking blood from the orbital plexus of rat and dissected for collection of the spleen, adrenal glands, thymus, and cotton pellets **Fig. 15** along with the clean extraneous tissue **Fig. 16**. Later the collected organs **Fig. 19** *i.e.,* spleen, adrenal gland, and thymus were preserved in a clean glass bottle containing 10% formaldehyde solution after proper weighing and send to the pathology laboratory for histopathological investigations. Also, the cotton pellet, along with extraneous tissue, was weighed (initial weight) and kept in a hot air oven overnight at 80 ºC for drying. Again the pellet was weighed after drying (final weight). The difference between the initial weight and final weight of the pellet was taken as the granuloma tissue weight. The result was expressed as mg granulation tissue formation per 100 g of body weight. The blood which was collected from the orbital plexus **Fig. 17** of rat separated from serum was analyzed for the determination of C-reactive protein. After sacrificing the animal's **Fig. 18**, the tissues were excised out immediately and were transferred into 10% Formaldehyde solution. The tissues were held in it till they are taken up of further processing (histopathological examination).

Acute Inflammatory Study:

FIG. 1: WEIGHING OF RATS

FIG. 2: GROUPING OF RATS

FIG. 3: PROVIDING FOOD & WATER

FIG. 4: APPLICATION OF OIL BEFORE EXP.

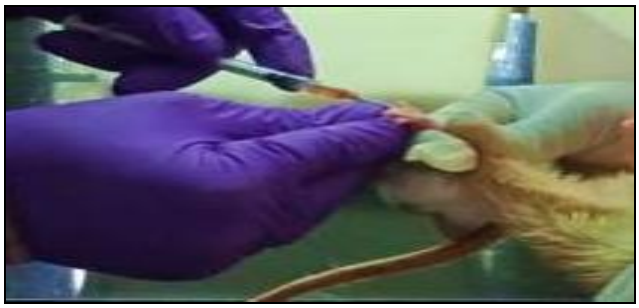


FIG. 5: INJECTING INFLAMMATION INDUCING AGENT

FIG 6. INFLAMMATION OF HIND PAW

FIG. 7: DIGITAL PLETHYSMOMETER

FIG. 8: READING OF PAW

Chronic Inflammatory Study:

FIG. 9: COTTON PELLET PREPARATION

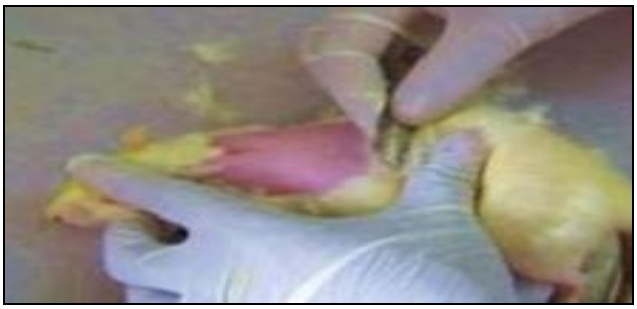


FIG. 10: PREPARATION FOR IMPLANTATION

FIG. 11: LOCAL ANAESTHESIA

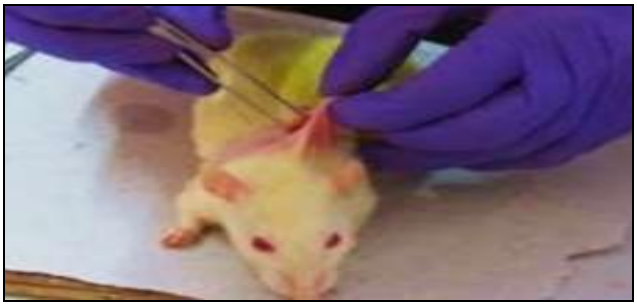


FIG. 12: COTTON PELLET IMPLANTATION

FIG. 13: SUTURING AFTER IMPLANTATION

FIG. 14: OIL FOR APPLICATION OVER SUTURED AREA

FIG. 15: REMOVAL OF COTTON PELLET

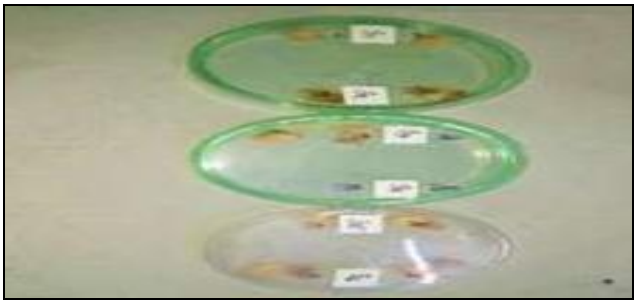


FIG. 16: COTTON PELLET GRANULOMATOUS TISSUE

FIG. 17: ORBITAL BLOOD FOR INVESTIGATION

FIG. 18: EUTHANASIA OF RAT

FIG. 19: ORGANS FOR HISTOPATHOLOGY

RESULT: The data were analyzed statistically using a one-way analysis of variance (ANOVA) followed by multiple comparison Dunnett's test as post hoc test.

The data shows there was a decrease in paw volume in the 24th hour in the standard group and test group when compared to the control group; the observed decrease was found to be statistically

non-significant. Thus in any acute condition, it can be used as an anti-inflammatory medicine as it is tridoshahara in nature and simultaneously pacify the signs and symptoms.

Acute Inflammatory Study: Data related to the effect of Murivenna on paw edema of carrageenan-induced paw edema at different time intervals is shown in **Table 3**.

The data shows there was a very significant increase in paw volume in the control group of 1st hour, 3rd hour and 6th hour and a non-significant increase in 24th hour when compared to the basal volume of the same group.

The data shows there was a very significant increase in paw volume in the castor control group of 1st hour, 3rd hour, 6th hour, and 24th hour when compared to the basal volume of the same group.

The data shows there was a very significant increase in paw volume in the standard group of 1st hour, 3rd hour, and 6th hour and a non-significant increase in 24th hour when compared to the basal volume of the same group.

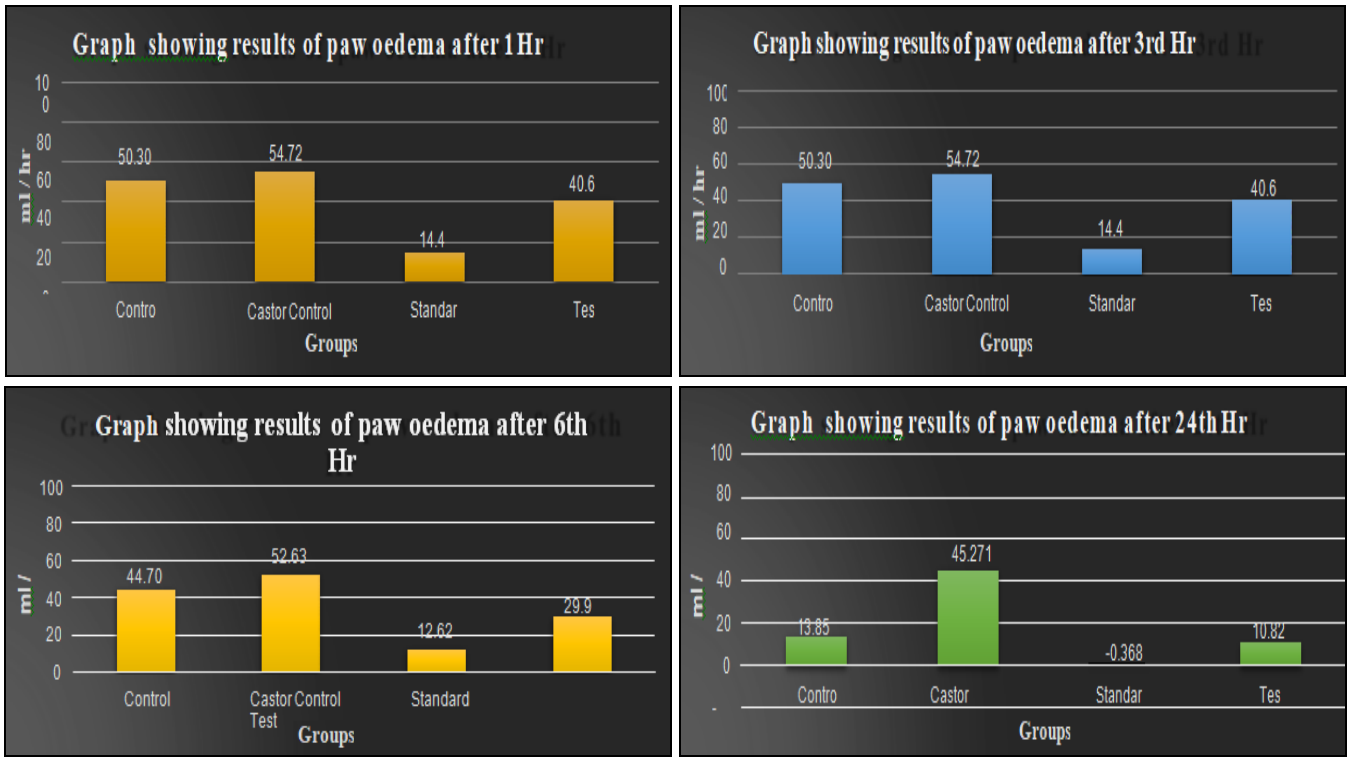
TABLE 3: THE EFFECT OF *MURIVENNA* ON PERCENTAGE INCREASE OF PAW OEDEMA AT DIFFERENT TIME INTERVAL

Mean ±SEM Percentage increase of paw oedema at different time interval					
Group	**Basal**	**1st hour**	**3rd hour**	**6th hour**	**24th hour**
Control	0.921±0.034	1.256±0.086**	1.665±0.030**	1.785±0.114**	1.073±0.045
Castor Control	0.818±0.024	1.153±0.048**	1.261±0.02**	1.245±0.03**	1.181±0.054**
Standard group	0.826±0.031	0.968±0.030**	0.941±0.023**	0.926±0.091**	0.823±0.030
Test group	0.66±0.0364	0.9±0.0304**	0.911±0.044**	0.84±0.021**	0.723±0.022

Data: MEAN ± SEM. **p < 0.01 in comparison to control group.

The data shows there was a very significant increase in paw volume in a test group of 1st hour, 3rd hour, and 6th hour and a non-significant increase in 24th hour when compared to the basal volume of the same group.

Acute Inflammatory Study Graph:



Chronic Inflammatory Study: The cotton pellet implanted granuloma method was used to evaluate chronic inflammation.

TABLE 4: EFFECT OF *MURIVENNA* ON COTTON PELLET IMPLANTED GRANULOMA FORMATION

Group	Mean ± SEM Granuloma tissue weight (mg)/100g body weight	% Change
Control	0.325 ± 0.03	-
Standard drug	0.172 ± 0.061	47.076 ↓
Test drug	0.303 ± 0.02	6.769 ↓

Data: MEAN ± SEM

Data related to the effect of test drug on the weight of cotton pellet has been shown in **Table 4**.

The data shows there was a decrease in cotton pellet implanted granuloma formation in the standard group and test group when compared to the control group; the observed decrease was found to be statistically non-significant.

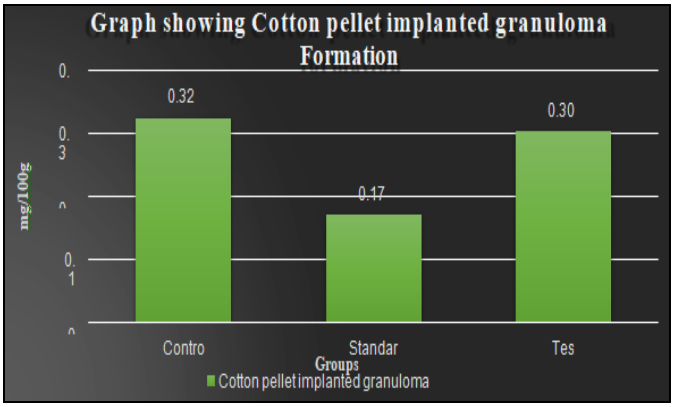


TABLE 5: THE EFFECT OF *MURIVENNA* ON WEIGHT OF SPLEEN IN COTTON PELLET IMPLANTED RATS

Group	Mean ± SEM Spleen weight (g)	% Change
Normal Control	0.976 ± 0.086	-
Control	1.185 ± 0.156	21.413 ↑
Standard drug	1.05 ± 0.118	7.581 ↑
Test drug	0.891 ± 0.087	8.709 ↓

Data: MEAN ± SEM

Data related to the effect of test drug on the weight of Spleen has been shown in **Table 5**.

The data shows there was an increase in weight of the spleen in the control group and standard when compared to the normal control group; the observed increase was found to be statistically non-significant.

The data shows there was a decrease in the weight of Spleen in the test group when compared to the normal control group; the observed decrease was found to be statistically non-significant.

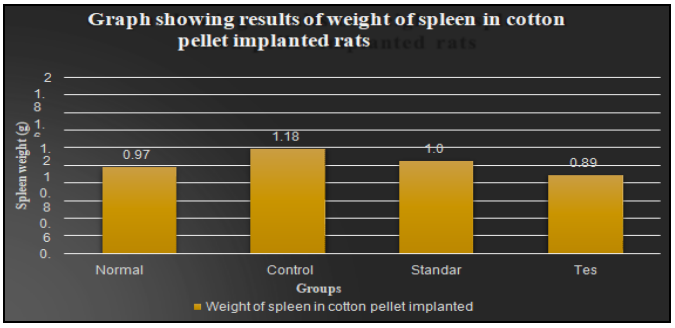


TABLE 6: THE EFFECT OF *MURIVENNA* ON WEIGHT OF THYMUS IN COTTON PELLET IMPLANTED RATS

Group	Mean ± SEM Thymus weight (g)	% Change
Normal Control	0.815 ± 0.141	-
Control	0.621 ± 0.033	23.803 ↓
Standard drug	0.583 ± 0.123	28.466 ↓
Test drug	0.593 ± 0.038	27.239 ↓

Data: MEAN ± SEM

Data related to the effect of test drug on the weight of Thymus has been shown in **Table 6**. The data shows there was a decrease in the weight of Thymus in the control group, standard group, and test group when compared to the normal control group; the observed decrease was found to be statistically non-significant.

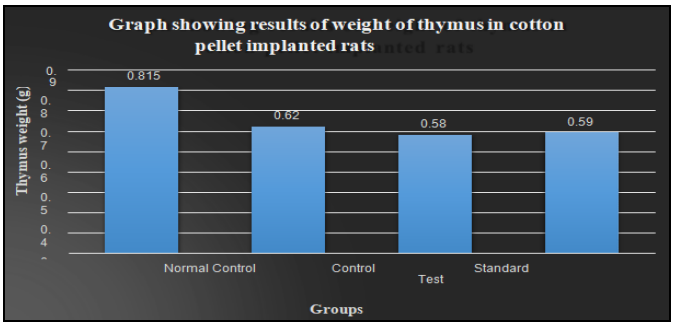


TABLE 7: THE EFFECT OF *MURIVENNA* ON WEIGHT OF ADRENAL GLAND IN COTTON PELLET IMPLANTED RATS

Group	Mean ± SEM Adrenal gland weight (g)	% Change
Normal Control	0.046 ± 0.008	
Control	0.106 ± 0.013	130.434 ↑
Standard drug	0.145 ± 0.045	215.217 ↑ *
Test drug	0.11 ± 0.013	139.130 ↑

Data: MEAN ± SEM, *p < 0.05 in comparison to normal control.

Data related to the effect of test drug on the weight of the Adrenal gland has been shown in **Table 7**.

The data shows there was an increase in the weight of the Adrenal gland in the control group and test drug when compared to the normal control group; the observed increase was found to be statistically non-significant.

The data shows there was an increase in the weight of the Adrenal gland in the standard group when compared to normal control group; the observed increase was found to be statistically significant.

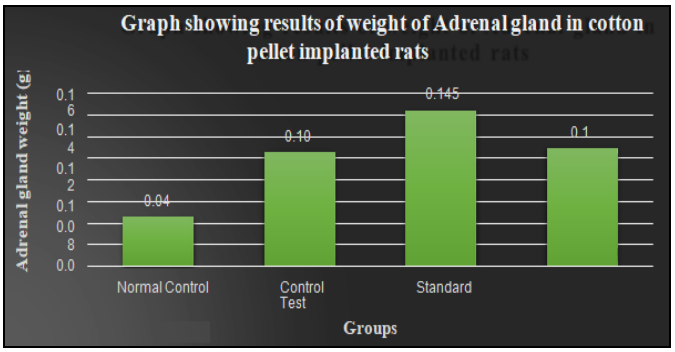


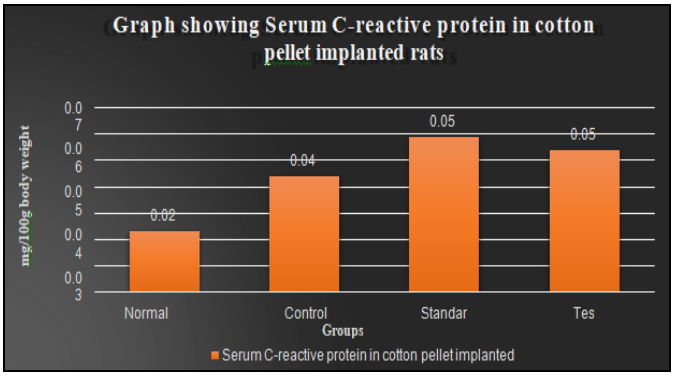
TABLE 8: THE EFFECT OF *MURIVENNA* ON SERUM C – REACTIVE PROTEIN LEVEL IN COTTON PELLET IMPLANTED RATS

Group	Mean ± SEM Granuloma tissue weight (mg)/100g body weight	% Change
Normal Control	0.023 ± 0.002	-
Control	0.044 ± 0.015	91.304 ↑
Standard drug	0.059 ± 0.016	156.521 ↑
Test drug	0.054 ± 0.029	134.782 ↑

Data: MEAN ± SEM

Data related to the effect of test drug on C-reactive protein has been shown in **Table 8**.

The data shows there was an increase in serum C – reactive protein in the control group when compared to the normal control group; the observed increase was found to be statistically non-significant.



The data shows there was an increase in C – reactive protein in the standard group and test group when compared to the normal control group; the observed increase was found to be statistically non-significant.

Result of Histopathology: Spleen:

TABLE 9: THE RESULT OF HISTOPATHOLOGY OF SPLEEN OF CONTROL GROUP (FIG. 22 & 23)

Rat no and section	Changes observed	Remarks
C1	White pulp increased. Disorganized white pulp. Abundance of hemosiderin pigment. Megakaryocytes present	Mild degenerative changes
C2	White pulp increased. Organized white pulp. Abundance of hemosiderin pigment	Normal
C3	White pulp increased. Pericapsular inflammation predominantly lymphocytes, plasma cells. Dilation of blood vessels, Disorganized white pulp, More inflammation in red pulp. Megakaryocytes present	Mild degenerative changes with severe inflammation
C4	White pulp increased and disorganized. More inflammation in red pulp. Megakaryocytes present	Mild degenerative changes with inflammation

TABLE 10: THE RESULT OF HISTOPATHOLOGY OF SPLEEN OF STANDARD GROUP (FIG. 24 & 25)

Rat no and section	Changes observed	Remarks
S1	Increased white pulp, sinusoidal enlargement. White pulp looking normal. Few megakaryocytes	Normal
S2	Increased white pulp. Dilation of blood vessels. Hemorrhage seen No megakaryocytes. Very less hemosiderin pigments	Normal
S3	Increased white pulp. Megakaryocytes seen. Abundance of hemosiderin pigments	Normal
S4	Severe necrosis. Change in architecture of white pulp. Megakaryocytes seen. Abundance of hemosiderin seen	Severe degenerative changes

TABLE 11: THE RESULT OF HISTOPATHOLOGY OF SPLEEN OF TEST GROUP (FIG. 26 & 27)

Rat no and section	Changes observed	Remarks
T1	Increased white pulp. Acute inflammation inside blood vessels. Change of tissue architecture in white pulp. No megakaryocytes. Hemosiderin pigment seen.	Mild degenerative changes with inflammation
T2	Increased white pulp. Inflammation inside blood vessels. Normal white pulp. Hemorrhage seen	Normal with Inflammatory changes
T3	Disorganized white pulp. Inflammation inside blood vessels. Megakaryocytes seen. Abundance of hemosiderin	Mild degenerative changes with inflammation. Extramedullary haematopoiesis.
T4	Increase in white pulp. Abundance of Megakaryocytes and hemosiderin pigment	Normal. Extra medullary haematopoiesis

Adrenal Gland:

TABLE 12: THE RESULT OF HISTOPATHOLOGY OF ADRENAL GLAND OF CONTROL GROUP (FIG. 30 & 31)

Rat no and section	Changes observed	Remarks
C1	Adrenal medulla shows hemorrhage and loss of architecture. Mild diffuse inflammation predominantly eosinophils. Slight hyperplasia of adrenal medulla.	Mild to moderate degenerative changes
C2	Severe necrosis in zona glomerulosa, Loss of nuclei in cells, Severe Haemorrhage, Acute inflammatory cells predominantly eosinophils, neutrophils around blood vessels, Pericapsular inflammation seen	Severe degenerative changes with inflammation
C3	Moderate tissue architecture change, focal necrosis in medulla, Haemorrhage, and mild inflammatory cells. Lipofuscin pigment in zona reticularis	Moderate degenerative changes
C4	Severe Haemorrhage with slight architectural change and mild inflammation	Mild to moderate degenerative changes

TABLE 13: THE RESULT OF HISTOPATHOLOGY OF ADRENAL GLAND OF STANDARD GROUP (FIG. 32 & 33)

Rat no and section	Changes observed	Remarks
S1	Tissue architecture almost normal. Slight Haemorrhage with mild inflammatory cells	Mild degenerative changes
S2	Vacuolization of cells seen. Loss of tissue architecture seen. Pericapsular inflammation seen	Mild to moderate degenerative changes with inflammation
S3	Almost normal tissue architecture. Dilated blood vessels.	No degenerative changes
S4	Almost normal tissue architecture. Hemorrhage with focal necrosis.	Mild degenerative changes

TABLE 14: THE RESULT OF HISTOPATHOLOGY OF ADRENAL GLAND OF TEST GROUP (FIG. 34 & 35)

Rat no and section	Changes observed	Remarks
T1	Moderate loss of tissue architecture. Vacuolization of cells seen. Lipofuscin pigment seen.	Moderate degenerative changes
T2	No loss of architecture.	No degeneration
T3	No loss of architecture. Focal necrosis. Enlarged nuclei in cells	Mild degeneration with regeneration
T4	Loss of tissue architecture with Haemorrhage seen in some areas. Pericapsular inflammation. Focal necrosis seen	Moderate degeneration

Thymus:

TABLE 15: THE RESULT OF HISTOPATHOLOGY OF THYMUS OF CONTROL GROUP (FIG. 38 & 39)

Rat no and section	Changes observed	Remarks
C1	Normal gland architecture with only few damaged ducts. No inflammation.	Mild degenerative changes
C2	Normal gland architecture with very few damaged acini and gland ducts. No inflammation	Mild degenerative changes
C3	Normal gland architecture with slight damage to few ducts	Mild degenerative changes
C4	Normal gland architecture with slight damage to few ducts	Mild degenerative changes

TABLE 16: THE RESULT OF HISTOPATHOLOGY OF THYMUS OF STANDARD GROUP (FIG. 40 & 41)

Rat no and section	Changes observed	Remarks
S1	Normal gland architecture. Small inflammatory infiltrate in some areas	Normal
S2	Normal gland architecture	Normal
S3	Normal gland architecture	Normal
S4	Normal gland architecture	Normal

TABLE 17: THE RESULT OF HISTOPATHOLOGY OF THYMUS OF TEST GROUP (FIG. 42 & 43)

Rat no and section	Changes observed	Remarks
T1	Normal gland architecture. Apoptotic change seen in some glands. No inflammation	Mild degenerative changes
T2	Normal gland architecture. No inflammation	Normal
T3	Normal gland architecture. Few damaged ducts seen	Mild degenerative changes
T4	Slight change in gland architecture	Mild degenerative changes

Photomicrograph of Histopathology Section: Spleen:

Normal control

FIG. 20: NC1

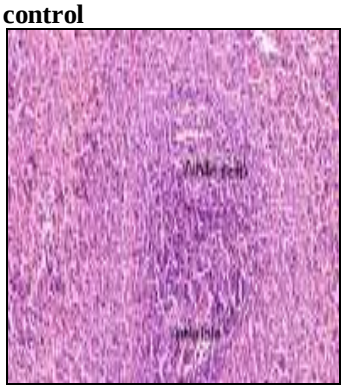


FIG. 21: NC2

Positive control

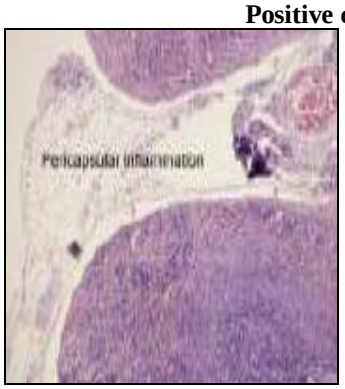


FIG. 22: PC1

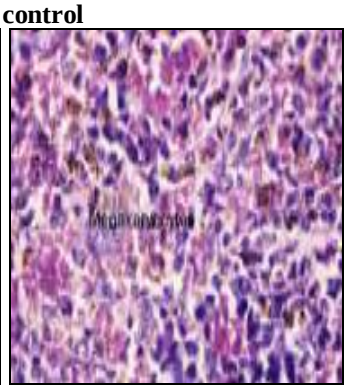


FIG. 23: PC2

Standard

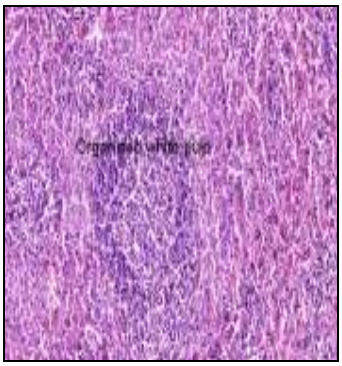


FIG. 24: STD1

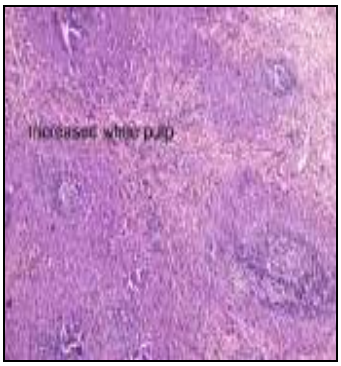


FIG. 25: DTD2

Test drug

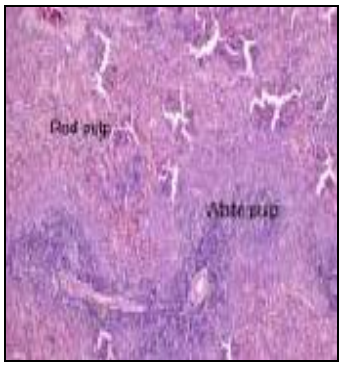


FIG. 26: T1

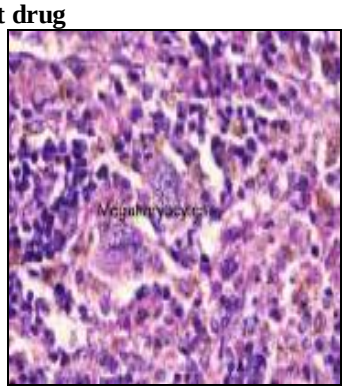


FIG. 27: T2

Adrenal:

Normal control

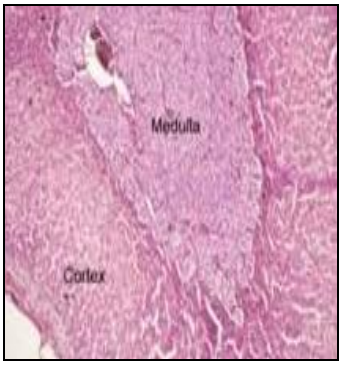


FIG. 28: NC1

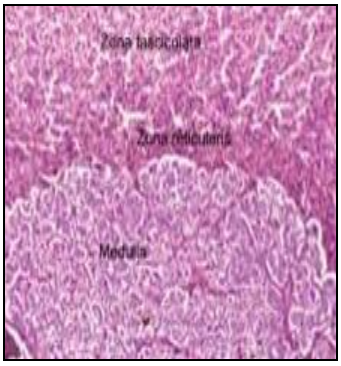


FIG. 29: NC2

Positive control

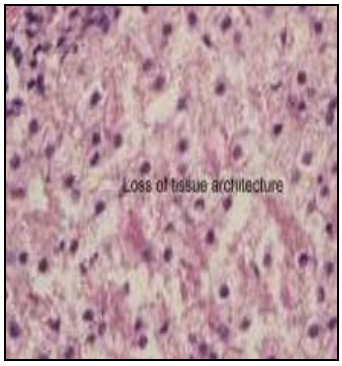


FIG. 30: PC1

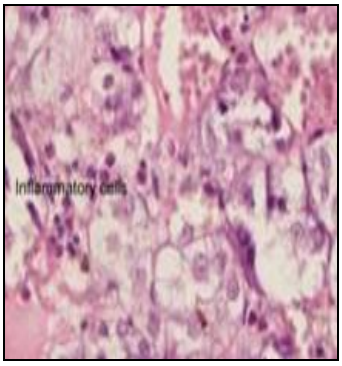


FIG. 31: PC2

Standard

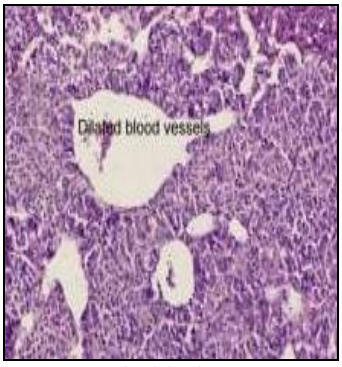


FIG. 32: STD1

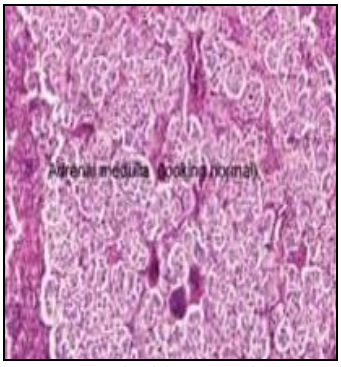


FIG. 33: DTD2

Test drug

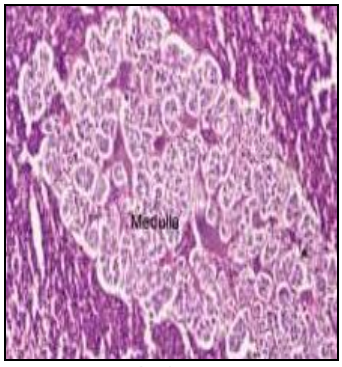


FIG. 34: T1

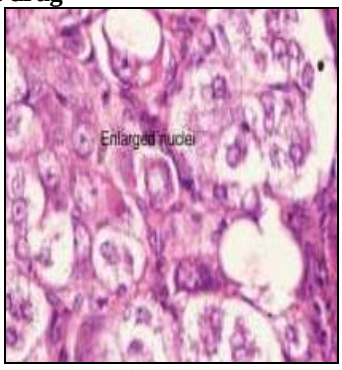


FIG. 35: T2

Thymus:

Normal control

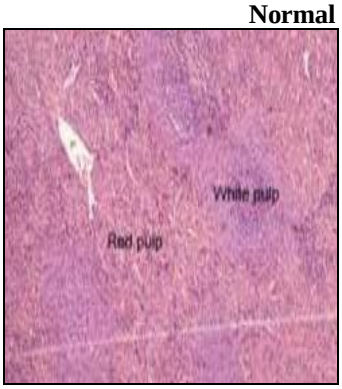


FIG. 36: NC1

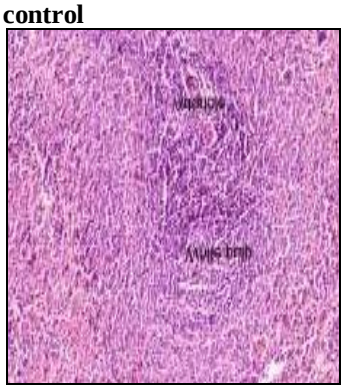


FIG. 37: NC2

Positive control

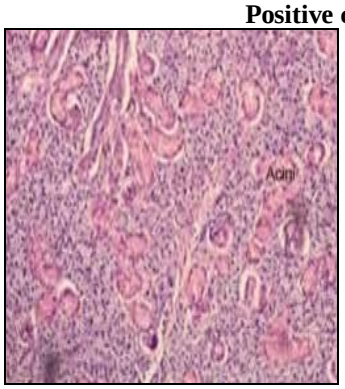


FIG. 38: PC1

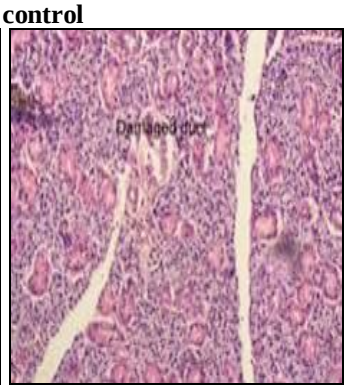


FIG. 39: PC2

Standard

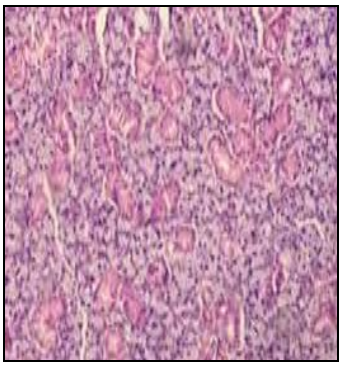


FIG. 40: STD1

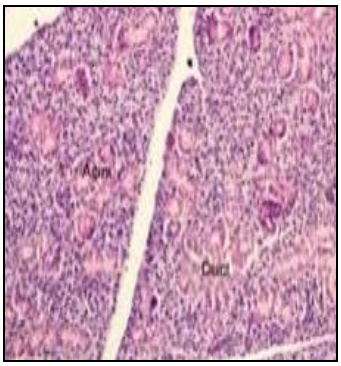


FIG. 41: DTD2

Test drug

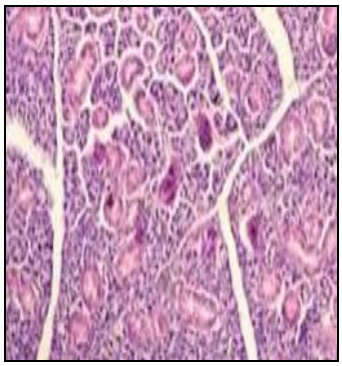


FIG. 42: T1

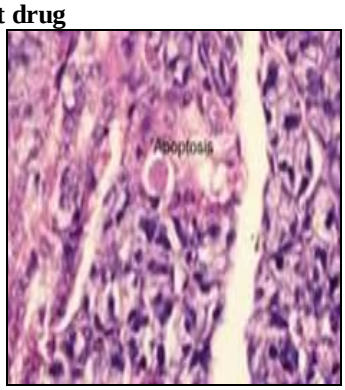


FIG. 43: T2

DISCUSSION:

TABLE 18: THE *MURIVENNA* INGREDIENTS WITH ANTI-INFLAMMATORY CONSTITUENTS

Sanskrit name	Botanical name	Part used	Anti-inflammatory constituents
Narikela taila	*Cocos nucifera*	-	Tryptophan, threonine, isoleucine, leucine, lysine, methionine, cysteine, phenylalanine, tyrosine, valine, arginine, histidine, alanine, aspartic acid, glutamic acid, glycine, proline serine
Tambula	*Piper betle*	Leaf (L.f.)	Eugenol, phenylalanine
Sigru	*Moringa oleifera*	Leaf (L.f.)	Agrinine, histidine, isoleucine, leucine, lysine, methionine, phenylalinine, threonine, tryptophan, valine, aspartic and glutamic acid
Paribhadra	*Erythrina indica*	Leaf (L.f.)	Alkaloids
Kanya	*Aloe barbadensis*	Leafpulp (L.f.Pp.)	Acetylated mannans, Poly mannans, C- glucosyl chromone, Fatty acids (4 plant steroids) – Cholestrol, Campestrol, Sisosterol, Lupeol (Antiseptic and Analgesic), Lignin – enhance penetrative effect of other ingredients into the skin. Salicylic acid (Anti-bacterial), Saponins – Cleansing and antiseptic property
Karanja	*Pongamia pinnata*	Bark (Bk)	-
Buka	*Spermacocea hispida*	Plant (Pl.)	Flavanoids, Beta- sitosterol, ursolic acid, isorhmnatin, saponins, tannins, phenolics, steroids, essential oils, flavonoids, terpenoids
Palaṇḍu	*Allium cepa*	Bulb(Bl.)	Quercetin, Oleoanolic acid, disulphide, allylpropyldisulphide, allicin, flavonoids, phenolic acids
Taṇḍulambu	*Oryza sativa*	Seed (Sd.)	-
Satavari	*Asparagus racemosus*	Root (Rt.)	Sarsapogenin, 2- spirostanolic and 2- furostanolic saponins, sitosterol

Discussion on Experimental Study: The experimental study of *Murivenna* has been designed to carry out the anti-inflammatory activity of the drug in an established animal model, as no work has been found reported in this formulation with specific to this activity. The outcome of experimental study has been provided in the form of consolidated tables as follows for easy comparison and discussion.

Discussion on Acute Inflammation:
Effect on Carrageenan Induced Paw Oedema:

TABLE 19: CONSOLIDATED STATEMENT ON PERCENTAGE CHANGE OF PAW OEDEMA AT DIFFERENT TIME INTERVAL ON ADMINISTRATION OF *MURIVENNA*

Time intervals	Castor control	Standard group	Test group
1st Hour	NSD	SD	NSD
3rd Hour	NSI	SD	NSD
6st Hour	NSI	SD	NSD
24th Hour	SI	NSD	NSD

Where NSD – Non-Significant Decrease, NSI – Non-Significant Increase, SI – Significant Increase

Carrageenan has been found to give results that are more consistent and is widely used as a standard edema-inducing agent. It is one of the most suitable acute models to screen anti-inflammatory agents. The edema developed in the paw of the rat after injection of carrageenan is a biphasic event. The initial phase of the edema is due to the release of histamine and serotonin, and the edema is maintained during the plateau phase by kinin like substance and the second accelerating phase swelling due to the release of prostaglandin like substances. In the present study, *Murivenna* is found to have non-significant decrease **Table 19** which means it significantly inhibited both the phases of carrageenan-induced paw edema.

Discussion on Chronic Inflammation: In chronic inflammatory model, the test formulation did not showed any significant impact on the majority of the parameters studied.

TABLE 20: CONSOLIDATED STATEMENT ON COTTON PELLET IMPLANTED GRANULOMA FORMATION ON ADMINISTRATION OF *MURIVENNA*

Parameter	Standard Group	Test Group
Granuloma Formation	NSD	NSD

Where NSD – Non Significant Decrease

Granulomatous tissue formation is related to the chronic inflammatory process, which is an indication for proliferative phases of inflammation. Inflammation involves the proliferation of macrophages, neutrophils, and fibroblast, which are the basic sources for the formation of granuloma. Thus this method is widely used to evaluate the transudative and proliferative components of chronic inflammation. The dry weight of the pellets relates to the amount of granulomatous tissue. Test formulation non-significantly decreased the weight of granulation tissue, and Diclofenac, which was used as a standard anti-inflammatory agent, also non- significantly decreased **Table 20** the weight of granulation tissue. This may indicate the ability of test formulation in reducing the synthesis of proteins, collagen, and infiltration of macrophages.

Discussion on Blood Parameter – CRP: CRP is present in the normal human body in minute amount. It is generally present in hepatocyte cells of the liver. It increases during any kind of inflammation or infection. CRP stimulates the immune system to produce more soldiers (neutrophils, lymphocyte, macrophages *etc*.) to fight against the condition. When antibodies are built sufficiently, CRP drops down. Thus here in this study, CRP is increased, showing inflammation in both test and standard group.

TABLE 21: CONSOLIDATED STATEMENT ON WEIGHT OF ORGANS ON ADMINISTRATION OF *MURIVENNA*

Organ weight	Control	Standard group	Test group
Spleen	NSI	NSI	NSD
Thymus	NSD	NSD	NSD
Adrenal Gland	NSI	SI	NSI

Where NSD – Non-Significant Decrease, NSI – Non Significant Increase, SI – Significant Increase

The weight of the spleen and thymus was non-significantly decreased in **Table 21** due to degenerative changes and the weight of the adrenal gland non significantly increased due to stimulation of organ activity.

Discussion on Histopathological Results: In a histopathological examination of spleen, thymus and adrenal gland sections, tissue architecture was normal in the normal control group. Mild to moderate degenerative changes with severe inflammation was noticed in the positive control group, and normal to mild changes found in the standard group. It is also noted that there are only mild degenerative changes found in the test group.

This indicates that the test formulation has a better effect in acute inflammation compared to chronic.

CONCLUSION: *Shopha* is compared to inflammation in contemporary science. As per Ayurveda, application of oil is indicated in s*hopha* or inflammation. *Murivenna* containing 10 main ingredients was prepared according to *Ayurvedic Formulary of India*, and an Experimental study was conducted in chemically and surgically induced inflammation in acute and chronic cases, respectively, in wistar albino rats.

The results indicate the presence of significant anti-inflammatory activity in the experimental model representing acute inflammation and non-significant effect in chronic inflammation. But there is a decrease in the granuloma formation, which suggests that *Murivenna* can be given in granuloma condition. The effects were statistically compared with the effect observed in control and the standard group, which indicate a significant effect in acute inflammation. Thus the obtained result clearly indicates *Murivenna* retains anti-inflammatory activity in acute inflammation than in chronic as most of the individual ingredients of the test formulation is proved for its anti-inflammatory action.

ACKNOWLEDGEMENT: I would like to express my sincere gratitude towards Mr. Sudhakar Bhatt - Research Officer, S.D.M Centre for Research in *Ayurveda* and Allied Science and Dr. Anjana Haridas, Dr. Jyothi K, Dr. Nisha G, Dr. Radhika Panicker, Dr. Sreeja Raj, Dr. Dhanya Krishnan, Dr. Priyada K.V, Dr. AkhilRaj. A.R, Dr. Chitra. S, Dr. Sai Lekshmi, Dr. Sujithra. M, Dr. Aswin T. Das- PG Scholars, Amrita School of Ayurveda, Kollam, Dr. Shruti Kamble – PG Scholar, MIAMS, Manipal, and Maya V. Nair, Venugopalan Nair K, Vaishakh V Nair, Kottayam for their valuable suggestions and support.

CONFLICTS OF INTEREST: We declare no conflicts of interest.

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How to cite this article:
Nair VV, Mohanan A and Mishra AK: An experimental study to evaluate anti-inflammatory activity of Murivenna in Wistar albino rats. Int J Pharm Sci & Res 2020; 11(10): 5091-03. doi: 10.13040/IJPSR.0975-8232.11(10).5091-03.