Journal of Pharmaceutical and Scientific Innovation

www.jpsionline.com (ISSN : 2277 -4572)

Research Article

STUDY OF THE ANTI-INFLAMMATORY ACTIVITY OF CRUDE DRUG FORMULATION WITH COTTON PELLETS INDUCED GRANULOMA IN RATS (CHRONIC INFLAMMATION)

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DOI: 10.7897/2277-4572.07177

Received on: 18/01/18 Revised on: 19/02/18 Accepted on: 26/02/18

ABSTRACT

The effect of crude drug anti-haemorrhoidal formulation on chronic inflammation in rats was studied. Cotton pellet-induced granuloma in rats is a sub-chronic model of inflammation and is sensitive to the treatment with the steroidal anti-inflammatory agents. The inflammatory reaction is readily produced in rats in the form of paw oedema with the help of irritants substances such as carrageenan, formalin etc. The animal model for anti-inflammatory activity (chronic inflammation) against the haemorrhoides used here is of cotton pellet induced granuloma in rats. Bark of *Berberis aristata*, Leaves and bark of *Cassia fistula*, Leaves of *Cynodon dactylon*, Fruits of *Emblica officinalis*, Leaves of *Tamarindus indica*, Fruits of *Terminalia chebula* and *Terminalia belerica*, Inflorescence of *Sphaeranthus indicus*, Bark and Leaves of *Syzigium cumini*, Bark of *Holarrhena antidysentrica* and Fruits of *Mesua ferrea* were the plant materials used in the preparation of crude drug formulation against haemorrhoides. Inflammation is one of the major part of haemorrhoides hence the effect of crude drug anti-haemorrhoidal formulation on the chronic inflammation was an essential aspect in the study. The conclusion of our study with this animal model (cotton pellet method) was thus that the Dose 3 (200 mg/kg) of the Crude anti-haemorrhoidal formulation was found to be the most effective dose for the anti-inflammatory activity.

Keywords: Anti-inflammatory, cotton pellet induced granuloma, chronic inflammation, haemorrhoides

INTRODUCTION

This model is based on the foreign body granuloma which is induced in rats subcutaneously by implantation of pellets of compressed cotton. After some days, giant cells, undifferentiated connective tissue and fluid infiltration can be observed histologically. The amount of newly formed connective tissue can be measured by weighing the dried pellets after removal. More exhaustive granuloma formation occurs if the cotton pellets have been impregnated with formalin.

In cotton pellet induced granuloma, foreign body like cotton or glass rod when implanted in the skin of animal produces undifferentiated connective tissue around it indicating state of inflammation. The amount of newly formed connective tissue is measured by weighing the dried pellet after removal as a proof of the severity of the inflammation. This model is the indication of the proliferative phase of the inflammation of the microphages, neutrophils, fibroblasts and collagen formation which are basic source for the granuloma formation; therefore decrease in the granuloma formation indicates the suppression of the proliferative phase[1].

The problem with the present day synthetic drugs is that they are toxic and reappearance of symptoms after discontinuation. Therefore, the screening and development of drugs for their anti-inflammatory activity is the need of an hour and there are many efforts for finding anti-inflammatory drugs from indigenous medicinal plants[2]. The use of NSAIDs is associated with many side effects, but their unwanted effects on the gastrointestinal tract, the kidney and the cardiovascular system are considered as major issues with the use of these drugs[3].

Principle: The cotton pellet-induced granuloma, described firstly by Meier, *et. al.* (1950), has been widely employed to assess the proliferative components of chronic inflammation[4]. Inflammation involves proliferation of macrophages, neutrophils and fibroblasts, which are basic source of proliferative components of chronic inflammation. In this method, foreign body granulomas are provoked in rats by subcutaneous implantation of pellets of compressed cotton. The inflammation and granuloma develops during the period of several days. Histologically, giant cells and undifferentiated connective tissue can be measured by measured by weighing the dried pellets after removal. The method has been useful for evaluation of steroidal and non-steroidal anti-inflammatory drugs. Steroids have the side effects of adrenal gland weight reduction. This method is also employed for measuring the adrenolytic properties of a steroid.

MATERIAL AND METHODS

Requirements

Animals: Albino rats weighing between 200 – 250 g of either sex were used for the study. They were kept in the Animal House of the college (Sharad Powar College of Pharmacy) under the standard environmental conditions of temperature (25 $\pm$ 2^{o}C), Humidity (55 $\pm$ 10%) and Light (12 : 12 h. Light/Dark cycle; lights on at 07 : 00 h). Rats were supplied with standard pellet diet (Goldmuhar Brand Rat Feed supplied by Lipton, India Ltd.) and

tap water ad libitum. The animals were handled and acclimatized to laboratory conditions 24 hours before conducting the experiments. All the experiments were conducted between 09: 00 and 18 : 00 hours. The parental administrations were given by disposable syringe and strict aseptic conditions were followed during the administration. The institutional animal ethics committee has approved the experimental protocols and was performed in accordance with the guidelines for the care and use of laboratory animals as adopted and promulgated by institutional animal ethical committee. (SPCP/2013/653-1 by CPCSEA).

Preparation/Plants used for the Formulation : 1 g crude drug contains *Berberis aristata* (90 mg), *Cassia fistula* (100 mg), *Cynodon dactylon* (80 mg), *Emblica officinalis* (120 mg), *Tamarindus indica* (80 mg), *Terminalia chebula* (100 mg), *Terminalia belerica* (100 mg), *Sphaeranthus indicus* (110 mg), *Syzigium cumini* (100 mg), *Holarrhena antidysentrica* (50 mg) and *Mesua ferrea* (70 mg).

Drugs: Dexamethasone (10 µg/pellet). Dissolve the drug in solvent (acetone) so as to contain 10 µg in 500µl of the solvent. Ethanol (70% v/v) as disinfectant and ethyl ether as an anesthetic. Keep all the solutions in ice and always prepare fresh solution.

Equipment: Surgical instruments, sterilized surgical cotton, analytical balance, oven, Tissue adhesive (insoluble sutures).

Procedure: Before proceeding towards the animal experiments, it was necessary to undertake the safety and efficiency of the formulation. Thus, the acute toxicity studies were conducted.

Acute toxicity studies: Acute oral toxicity studies were carried out according to OECD guidelines 423. The animals of both sexes were selected by random sampling technique and divided into 5 groups of 3 animals each. A single oral dose (200, 400mg, 600mg, 800mg and 1000mg/kg) of each extract was administered orally at the dose level up to 1000mg/kg body weight. The animal groups were observed for appearance of toxic symptoms including behavioral changes, locomotion, muscle spasm, loss of righting reflex, tremor, convulsions and mortality for 24 h and further supervised for a period of 14 days for occurrence of toxic symptoms and mortality. However, from the first day till the 14th one, there were no such adverse symptoms as mentioned above. There was no change in their behavior or their living skills also, infact they remained unaffected completely. However, a bit of sluggishness was observed at higher doses.

The animals were weighed and numbered. They were randomized into different groups (Vehicle/Standard/Crude Drug) according to the body weights. Each group consisted of six animals. The hands were washed with disinfectant to maintain the aseptic conditions. Cotton pellets (each weighing 20 $\pm$ 1 mg weight) were prepared by weighing the raw cotton on a weighing balance and then were rolled in a pellet shape. These pellets were sterilized in an oven and placed individually in a container/petridish. 500µl (10 µg of drug/500µl of acetone) of the vehicle/Drug/Standard solution was applied to the respective pellets and were allowed to dry at room temperature to achieve the constant weight.
The animals were anaesthetized by using ethyl ether. The animal was procured on the dissecting board and its back was shaved off. The shaved area was cleaned with 70% ethanol as disinfectant and an incision was given with the help of surgical blade in the lumbar region of the rat. By using the artery forceps, subcutaneous tunnels were formed to place the sterilized cotton pellets (treated with vehicle/Standard/Crude Drug) in the scapular region. The incision was sealed with the insoluble sutures. The cotton pellets were allowed to remain in the body for six days.

On the seventh day, the animals were weighed again and sacrificed by using ethyl ether anesthesia and the pellets were removed. The adrenals were also removed with the help of forceps and scissors and were separately weighed. This pellet weight referred to the wet weights of granuloma plus weight of the cotton implanted. The pellets were then allowed to dry in an oven at 60^{0}C for 18 hours. The pellets were removed from the oven and weighed again. This referred to the dry weight of the granuloma plus weight of the cotton implanted. The individual weights of the cotton (20$\pm$1 mg) were subtracted from the above mentioned weights. This gave the respective net wet or dry weights of the granuloma. It was then compared with the Vehicle treated group. The percentage change in the body weight on the seventh day as compared to the first day was calculated.

$$\text{Adrenal weight/100 g BW} = \frac{\text{Weight of the Adrenal Gland X 100}}{\text{Body Weight on 7}^{th}\text{ day}}$$

$$\% \text{ increase or decrease} = \frac{A - B}{A} \times 100$$

Where, A = Mean Weights of Adrenal/ 100g BW of Vehicle treated group
B = Mean Weights of Adrenal/ 100g BW of Dexamethasone treated group

The difference between the initial weight and the final weight of the cotton pellet is the amount of granulation tissue formed[3]. The percentage inhibition of granulation tissue formation was measured by the following formula:

$$\% \text{ Inhibition} = \frac{(X - Y)}{X} \times 100$$

Where, X = mean increase in cotton pellet weight of rats in the control group
Y = mean increase in cotton pellet weight of rats in the drug treated group

Experimental Design

Treatment Groups: The animals were divided into different groups and each group consists of six animals. All the doses and the standard Dexamethasone were prepared as mentioned above.

Group 1 (Control Group): Rats were treated with normal saline solution.

Group 2 (Standard Group): Rats were treated with Dexamethasone 500µl (10 µg of drug/500µl of acetone) through the intra-peritoneal route.

Group 3 (Dose 1Group): Rats were treated with Dose 1 orally (50 mg/kg/day) of the anti-haemorrhoidal crude drug formulation.

Group 4 (Dose 2 Group): Rats were treated with Dose 2 orally (100 mg/kg/day) of the formulation.

Group 5 (Dose 3 Group): Rats were treated with Dose 3 orally (200 mg/kg/day) of the formulation.

OBSERVATIONS

Table 1: Effect of Dexamethasone on Body Weight of Albino Rats

Sr. No.	Treatment	Body Weight (g) on Day 1	Body Weight (g) on Day 7	% Increase in Body Weight
1.	Vehicle Control	155.125± 2.46	193.25 ± 2.42	24.5 ± 0.64
2.	Standard - Dexamethasone (10 µg/Pellet)	160.85 ± 6.66	180.05 ± 6.21	12.25 ± 1.43
3.	Dose 1 (50 mg/kg)	147.75 ± 1.54	176.375± 3.48	19.69 ± 2.62
4.	Dose 2 (100 mg/kg)	150.8 ± 2.91	174 ± 3.48	16.89 ± 2.36
5.	Dose 3 (200 mg/kg)	158.3 ± 1.34	178.2 ± 3.48	12.57 ± 1.83

Note: Values are expressed as Mean Values ± Standard Error at N = 6, (p <0.5).

Table 2: Effect of Dexamethasone on Wet Weight Granuloma Formation in Albino Rats

Sr. No.	Treatment	Weight Of Cotton Implanted (mg)	Weight of Pellet Removed (mg)	Wet Weight of Granuloma (mg)	% Decrease in Wet Weight of Granuloma	% Inhibition
1.	Vehicle Control	19.75 ± 0.32	221 ± 0.57	201.75 ±0.47	91.29 ± 0.1	-
2.	Standard Dexamethasone (10 µg/Pellet)	21.5 ± 2.19	118.54± 0.72	97.04± 1.03	81.86 ± 1.47	51.90
3.	Dose 1 (50 mg/kg)	19.11 ± 2.19	149.6 ± 0.72	130.49± 1.03	87.22 ± 0.62	35.32
4.	Dose 2 (100 mg/kg)	20.5± 1.03	128.96± 1.03	108.46± 1.03	84.10± 1.03	46.24
5.	Dose 3 (200 mg/kg)	20.5 ± 1.28	119.25 ± 0.47	98.75± 0.75	82.80 ± 0.76	51.05

Note: Values are expressed as Mean Values ± Standard Error at N = 6, (p <0.5).

Table 3: Effect of Dexamethasone on Dry Weight Granuloma Formation in Albino Rats

Sr. No.	Treatment	Weight Of Cotton Implanted (mg)	Weight of Dried Pellet (60^0 C for 18 hours (mg)	Dry Weight of Granuloma (mg)	% Decrease in Dry Weight of Granuloma	% Inhibition
1.	Vehicle Control	19.75 ± 0.32	72.25 ± 0.85	52.5 ± 0.28	72.66 ± 0.89	-
2.	Standard Dexamethasone (10 µg/Pellet)	21.5 ± 2.19	62.25 ± 0.85	40.75 ± 1.22	65.46 ± 1.88	22.38
3.	Dose1(50 mg/kg)	19.11 ± 2.19	65.5 ± 3.06	46.39 ± 0.85	70.82 ± 3.98	11.63
4.	Dose2(100 g/kg)	20.5± 1.03	64.35 ± 0.26	43.85 ± 1.47	68.14 ± 1.63	16.47
5.	Dose3(200 g/kg)	20.5 ± 1.28	62.33 ± 0.40	41.83 ± 2.35	67.11 ± 1.48	20.32

Note: Values are expressed as Mean Values ± Standard Error at N = 6, (p <0.5).

Table 4: Effect of Dexamethasone on Adrenal Gland in Albino Rats (Wet Weight)

Sr. No.	Treatment	Weight Of Animal on 7^{th} Day (mg)	Weight of Adrenal Glands (mg)	Weight of Adrenal per 100g of Body Weight (mg)	% Decrease in Adrenal Glands
1.	Vehicle Control	193.25 ± 0.08	58.97 ± 1.69	30.51 ± 0.84	-
2.	Standard Dexamethasone (10 µg/Pellet)	180.05 ± 0.78	42.125 ± 1.38	23.39 ± 1.05	23.36 ± 1.88
3.	Dose 1(50 mg/kg)	176.375± 3.48	81.25 ± 0.47	46.06 ± 0.24	50.96 ± 2.59
4.	Dose 2 (100 mg/kg)	174 ± 0.88	80.87 ± 0.86	46.47 ± 1.55	52.31 ± 2.16
5.	Dose 3 (200 mg/kg)	178.2 ± 0.19	84.10 ± 2.16	47.19 ± 2.73	54.67 ± 1.38

Note: Values are expressed as Mean Values ± Standard Error at N = 6, (p <0.5).

Table 5: Effect of Dexamethasone on Adrenal Gland in Albino Rats (Dry Weight)

Sr. No.	Treatment	Weight Of Animal on 7th Day (mg)	Weight of Adrenal Glands (mg)	Weight of Adrenal per 100 g of Body Weight (mg)	% Decrease in Adrenal Glands
1.	Vehicle Control	193.25 ± 0.08	38.875 ± 5.22	20.11 ± 2.52	-
2.	Standard Dexamethasone (10 µg/Pellet)	180.05 ± 0.78	27.27 ± 5.42	15.14 ± 2.51	24.71 ± 2.67
3.	Dose 1 (50 mg/kg)	176.375± 3.48	61 ± 1.5	34.58 ± 1.39	71.95 ± 2.59
4.	Dose 2 (100 mg/kg)	174 ± 0.88	60.83 ± 1.39	34.95 ± 1.12	73.79 ± 1.83
5.	Dose 3 (200 mg/kg)	178.2 ± 0.19	62.34 ± 2.92	34.98 ± 1.28	73.94 ± 1.56

Note: Values are expressed as Mean Values ± Standard Error at N = 6, ($p < 0.5$).

RESULTS AND DISCUSSION

As mentioned in Table 1., there was a significant increase in the body weight of the rats from Day 1 to Day 7 in all the experimental animals. The increase in the body weight of the animals treated with the crude drug anti-haemorrhoidal formulation Dose 1(50 mg/kg), Dose 2(100 mg/kg) and Dose 3 (200 mg/kg) was 19.69 ± 2.62%, 16.89 ± 2.36% and 12.57 ± 1.83% respectively. These values were between the values of Vehicle Control (24.5 ± 0.64%) and Standard Dexamethasone (12.25 ± 1.43%).

There was a % decrease in Wet weight of Granuloma in each case. In Vehicle Control, it was 91.29 ± 0.1% while in Standard Dexamethasone it was 81.86 ± 0.76%. The wet weight of granuloma of crude drug anti-haemorrhoidal formulation Dose 1, Dose 2 and Dose 3 treated animals was 87.22 ± 0.62%, 84.10 ± 1.03% and 82.80 ± 0.76% respectively. The value of the third Dose of the crude drug anti-haemorrhoidal formulation for the mentioned context was close to that of the Vehicle Control and in between of Vehicle Control and The Standard Dexamethasone. Thus, Dose 3 shows the maximum % decrease in Wet weight of Granuloma.

The results of the formation of granulation tissue (Wet weight of Granuloma) around the transplanted sterilized cotton in the lumbar region of the rat have been shown in Table 2. The mean values of each group were taken and the percentage inhibition of granulation tissue formation calculated with reference to the control group. Overall, the standard drug Dexamethasone showed more inhibition of granuloma formation (51.90%) than the different doses of crude drug anti-haemorrhoidal formulation. The 200 mg/kg dose of the crude anti-haemorrhoidal formulation showed 51.05 % inhibition when compared with the control group (table 2). Similarly, the 100 mg/kg and 50 mg/kg dose of crude drug anti-haemorrhoidal formulation showed 46.24 % and 35.32% inhibition respectively during the study. In terms of overall anti-inflammatory effect, the crude drug anti-haemorrhoidal formulation exhibited highly significant impact of 200 mg/kg dose which was close to that of the standard drug and a slightly lower efficacy at the dose of 100 mg/kg. Among the three doses of the crude drug evaluated during the study, the third one (200 mg/kg) exhibited the highest inhibition of granulation tissue formation followed by a slightly lower impact in second dose (100 mg/kg) and the lowest effect in case of the dose 1 (50 mg/kg).

The results of the formation of granulation tissue (Dry weight of Granuloma) i.e. after drying the pellet at 60°C for 18 hours, have been shown in Table 3. Here also, the mean values of each group were taken and the percentage inhibition of granulation tissue formation calculated with reference to the control group. The standard drug Dexamethasone showed more inhibition of granuloma formation (22.38%) than the different doses of crude drug anti-haemorrhoidal formulation. The 200 mg/kg dose of the crude anti-haemorrhoidal formulation showed 20.32% inhibition when compared with the control group (Table 3). Similarly, the 50 mg/kg and 100 mg/kg doses the of crude drug anti-haemorrhoidal formulation showed 11.63% and 16.47% inhibition respectively during the study. The crude drug anti-haemorrhoidal formulation exhibited high anti-inflammatory activity at 200 mg/kg dose which was close to that of the standard drug and a slightly lower efficacy at the dose of 100 mg/kg. Among the three doses of the crude drug evaluated during the study, the third one (200 mg/kg) exhibited the highest inhibition of granulation tissue formation followed by a slightly lower impact in second dose (100 mg/kg) and the lowest effect in case of the dose 1 (50 mg/kg) as in case of the Wet Weight Granulation studies. Thus, the results are more or less similar in both the cases i.e. the anti-inflammatory activities of the crude drug anti-haemorrhoidal formulation of dose 3 (200 mg/kg) was comparatively higher than the other two doses (100 mg/kg and 50 mg/kg respectively).

As mentioned in Table 4., there was a significant decrease in the Wet weights of the Adrenal Glands also in the rats from Day 1 to Day 7 in all the experimental animals. The weight of the Adrenals per 100 g of the Body Weight in Vehicle Control, Standard Dexamethasone and Dose 1 (50 mg/kg) of the crude anti-haemorrhoidal formulation was found to be 30.51 ± 0.84 mg, 23.39 ± 1.05 mg and 46.06 ± 0.24 mg respectively. In case of Dose 2 (100mg/kg) and Dose 3 (200mg/kg) treated animals, the values were 46.47 ± 1.55 mg and 47.19 ± 2.73 mg respectively. The % decrease in the weight of Adrenal Glands (Wet) in case of Doses 1, 2 and 3 of the crude drug anti-haemorrhoidal formulation treated animals was 50.96 ± 2.59%, 52.31 ± 2.16%, and 54.67 ± 1.38% while of the Standard Dexamethasone, it was 23.36 ± 1.88% as compared to the Vehicle Control.

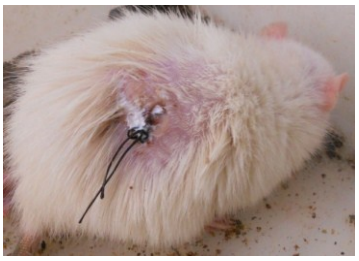
Table 5., shows the decrease in the Dry weights of the Adrenal Glands in the rats from Day 1 to Day 7. The weight of the Adrenals per 100 g of the Body Weight in Vehicle Control, Standard Dexamethasone and Dose 1 (50 mg/kg) of the crude anti-haemorrhoidal formulation was found to be 20.11 ± 2.52 mg, 15.14 ± 2.51 mg and 34.58 ± 1.39 mg respectively. In Dose 2 (100

mg/kg) and Dose 3 (200 mg/kg) treated animals, it was 34.95 ± 1.12% and 34.98 ± 1.28% respectively. The % decrease in the weight of Adrenal Glands (Dry) in case of Dose 1, Dose 2 and Dose 3 treated animals was 71.95 ± 2.59%, 73.79 ± 1.83% and 73.94 ± 1.56% respectively while of the Standard Dexamethasone, it was 24.71 ± 2.67% as compared to the Vehicle Control. Thus, all the mentioned results suggest Dose 3 (200 mg/kg) of the Crude anti-haemorrhoidal formulation to be the most effective dose for the anti-inflammatory activity.

CONCLUSION

Cotton pellet-induced granuloma in rats is a sub-chronic model of inflammation and is sensitive to the treatment with the steroidal anti-inflammatory agents. The dexamethasone showed decrease in wet as well as dry weight granuloma as compared to vehicle control group and thus has anti-inflammatory property. Same was the case with Dose 3 (200 mg/kg) of the crude drug anti-haemorrhoidal formulation and it showed results almost next to that of the Standard. Dexamethasone showed adrenolytic property while it was not exhibited by the formulation.

Cotton –pellet Granuloma Formation



Zero day

Seventh day, before sacrificing

ACKNOWLEDGEMENTS

The authors are thankful to Mrs. Shilpa Deshpande (Sharad Pawar College of Pharmacy) for providing all the necessary facilities as well as permission for conducting the animal experiments in their institution.

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How to cite this article:
Samiksha P. Dandekar and Dayanand P. Gogle. Study of the anti-inflammatory activity of crude drug formulation with cotton pellets induced granuloma in rats (chronic inflammation). J Pharm Sci Innov. 2018;7(1):28-32.
http://dx.doi.org/10.7897/2277-4572.07177

Source of support: Nil, Conflict of interest: None Declared