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SHORT COMMUNICATION

Study on Formulation, Standardization of Herbal Suspension Containing *Musa granatum* and its Efficacy against Carrageenan Induced Prostatitis in Rodent Model

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Abstract

Prostatis prevalence more in aged males and amidst disputes of severe therapeutic measures, both physicians and patients resort to alternative and non-traditional measures. The aim of this study is to investigate the effect of *Musa paradisiaca* (lyophilized juice) and *Punica granatum* (peel powder) suspension by carrageenan-induced method for prostatitis in Sprague Dawley rats. Suspension of *Musa paradisiaca* (lyophilized juice) and *Punica granatum* (peel powder) were prepared in equal proportion with high dose (400 mg/kg) and low dose (200 mg/kg) after carrageenan induction for one week. The body weight variation, urine volume analysis, white blood cell count in blood, net weight of the prostate gland and histopathological analysis were the parameters assessed. *Punica granatum* shows a significant increase in body weight, Prostate gland weight and white blood cell count and a decrease in urine volume in the prostatitis-induced control group animals. In *Musa paradisiaca,* a decrease in body weight, an increase in the prostate gland, white blood cell count and weight in urine volume was observed. The histopathological report evidence that the presence of leukocytes infiltration, papillary fronds were present and an increase in epithelial height was observed in the carrageenan-induced control group. It has been concluded that the low dose suspension of *Musa granatum* in 200 mg/kg has given a significantly better amelioration effect than compared to other intervention groups.

Keywords: *Punica granatum*, *Musa paradisiaca* Suspensions, Prostatitis, Prostate Inflammation

1. Introduction

Multifactorial factors have been attributed to prostatitis where inflammation of the prostate gland causes pain difficulty in urination, and obstruction of the vas deferens. Nearly 50% of the male population at the age of 60 and above suffers from benign prostatic hyperplasia. Prostatitis left untreated leads to permanent infertility and also increases in size and hardening of the prostate gland cause of prostate cancer in the advanced stages. Amidst disputes over several therapeutic measures, both physicians and patients resort to alternative and non-traditional measures[1].

Punica granatum a predominant member of the Punicaceae family has proven to exhibit important physiological properties, such as anticancer[2,3] anti-proliferative, apoptotic[4] HIV-I entry inhibitor[5] cardioprotective, antihyperlipidemic, anti-inflammatory, anti-mutagenic, anti-bacterial activities and as a powerful antioxidant and antifungal substance[6,7].

Musa paradisiaca is used traditionally for treating diarrhoea, diabetes in sprue, uremia, nephritis, gout, hypertension, cardiac disease, dysentery, cholera, otalgia and also its role against hypertension, hypoglycemia, atherosclerosis, wound healing, malaria, anti-microbial and infertility has been proved scientifically[8].

2. Materials and Methods

2.1 Procurement of Experimental Animals

Adult male Sprague Dawley strain procured from authorized breeders at Bangalore and housed as per CPCSEA guidelines (IAEC/196/2018), the rats weighed from 150 to 200 gm.

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2.2 Preparation of Suspension

All the plant parts of Musa and pomegranate were collected from the local market, and identification of *Musa paradisiaca* and *Punica granatum* was done at the Plant Anatomy Research Centre (PARC), Tambaram, Chennai, India.

Musa paradisiaca bark region is collected, the white inner part of the stem is cut down into pieces, and it is crushed by a mechanical crusher for the extraction of fresh juice. About 5 litres of fresh juice is collected and filtered. About 150 ml of juice is lyophilized to get a gummy material[8]. Pomegranate (*P. granatum*) peel was collected, and shade-dried until it was brittle and easy to break into pieces. The pomegranate rind was powdered finely and sieved further to acquire a uniform size by using a sieve size number[9]. The lyophilized stem juice of *M. paradisiaca* and grind powder of *P. granatum*, prepared individually, were suspended in water using tween- 80 (suspending agent). Sodium benzoate and lemon oil according to the quantity mentioned in the Table 1. The suspension was named as *Musa granatu*m (combination of *Musa paradisica* and *Punica granatum*).

2.3 Stability Parameters for Suspension

The Physical Test of formulation was analysed based on parameters like sedimentation volume, re-dispersibility, flow rate, viscosity, pH and crystal growth, and all were performed based on standard protocol[10].

2.4 Chemical Compatibility Test

The functional group analysis and Chemical compatibility test were performed by Fourier Transform Infrared Spectrophotometer (IRTracer-100) SHIMADZU[11].

Table 1. *P. granatum* suspension formulation

S. No.	Name of the Ingredient	Low Dose 200 mg	High Dose 400 mg
1	*M. paradisiacal* stem juice lyophilized	8 gm	16 gm
2	*P. garantum* rind powder	4 gm	8 gm
3	Tween – 80	0.2%w/v	0.2%w/v
4	Sodium benzoate	0.2 gm	0.2 gm
5	Lemon oil	2 ml	2 ml
6	Sodium CMC	2.0%	2.0%
7	Purified water q.s	200 ml	200 ml

CMC - Carboxymethyl cellulose

2.5 Carrageenan Induction

Carrageenan is a polysaccharide an inflammatory-inducing agent which triggers the inflammatory mediators and causes inflammation according to the triple response of Lewis.

Sprague Dawley rats were randomly divided into five groups. The lower abdomen region just above the penis was depleted prior to injection, and the depleted skin was sterilized using applications of 70% v/v ethanol followed by 10% povidone-iodine solution thrice. A one-inch midline incision was made in the sterilized area, and then the bladder, prostate lobes and seminal vesicles were exposed (Figure 1). 50 µl of sterile intraprostatic injection of 3% carrageenan suspension was administered on either side of the prostate lobe using a gauge needle, (group, n = 6). Topical application of a 2% lidocaine gel was applied to the exposed region, and then the cut open was closed by using non-absorbable surgical suture #3.0 with sterilized surgical needled suture followed by topical neomycin antibiotic cream used[12].

2.6 Grouping and Treatment

Experimental animals were divided into five groups of 6 animals in each group. The low and high dose of poly herbal suspension was administered orally for Groups IV and V. Except for Group I in all other groups prostatitis is induced by carrageenan intraprostatic injection. Group III was treated with fresh juice of both test extracts orally. The treatment schedule was

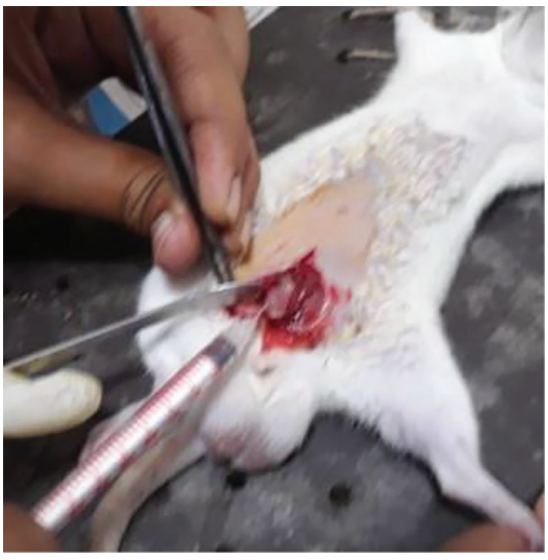

Figure 1. Carrageenan induction Sprague Dawley rats.

Table 2. Physical tests for *Musa granatum* suspension

S. No.	Parameter	Initial		Room temperature		45°C	
		F_{LD}	F_{HD}	F_{LD}	F_{HD}	F_{LD}	F_{HD}
1.	Nature	Liquid	Liquid	Liquid	Liquid	Liquid	Liquid
2.	Colour	Yellowish green	Greenish brown	Yellowish Green	Greenish brown	Yellowish green	Greenish brown
3.	Odour	Pleasant	Pleasant	Pleasant	Pleasant	Pleasant	Pleasant
4.	Texture	Suspension	Suspension	Suspension	Suspension	Suspension	Suspension

continued for seven days. The rats were provided with free access to feed and water during the test procedure.

2.7 Parameters Evaluated

2.7.1 Urine Analysis

The animals were placed in a metabolic cage, and the urine excreted by the animals was collected using a beaker. The volume of urine excreted by the animal was recorded at 3 hr and 5hrs after placing the animals in the metabolic cage individually.

2.7.2 Body Weight

The body weight of each animal was examined prior to induction of the disease and assessed during the intervention period and final day of study.

2.7.3 Hematological Parameters

2.7.3.1 WBC Count Analysis

WBC count analysis was examined on the final day of the intervention period. The blood of the animals was obtained by the retro-orbital plexus puncture, and the total count of WBC was recorded.

2.7.4 Prostate Weight Assessment

The prostate gland was carefully dissected by removing the urinary bladder and the seminal vesicles and was weighed.

2.7.5 Histopathological Evaluation

The prostate gland was carefully dissected. The dissected prostate gland was immersed in a 10 % formaldehyde solution and subjected for evaluation.

3. Results and Discussion

Musa paradisica and *Puna granulatum* were mixed to prepare the suspension physicochemical stability is an essential parameter in suspension development. The formulation prepared is yellowish green and greenish-brown in color, respectively low dose and high suspension, liquid in nature, pleasant odour with a characteristic taste. The sedimentation volume is 1, which indicates the formulation is in acceptable form[13]. In Table 2, the organoleptic properties of the formulation are mentioned. Also in Table 3, the pH, Redispersibility, flow rate and viscosity of the suspension are represented. The suspension crystal formation related to time duration at particular temp of other low and high doses is represented in Table 4. In Table 5 sedimentation rate of the suspension is mentioned which indirectly reveals that no flocculation was observed.

3.1 Stability Parameters for Suspension

It has the optimum viscosity, which has the tendency of easily pourable and also established the shear thickening effect. It is observed that suspension of these formulations was found to be stable at different temperatures, and no crystal growth was found in the evaluation. The stability study testing of the *Musa granatum* suspension in a low dose of 200 mg/kg and a high dose of 400 mg/kg was performed. The formulation in the form of suspension contains preservatives, flavoring agents, thickening agents and suspending agents. Tween 80 is a polysorbate surface active agent to increase the bioavailability of the oral suspension as it is non-ionic in nature so it does not alter the pH range

Table 3. Accelerated stability studies

S. No.	Parameter	F_{LD}	F_{HD}
1	Redispersibility	1 inversion	1 inversion
2	pH	7.4	7.8
3	Flow rate	5ml/52 s	5ml/59 s
4	Viscosity	67.8cP	74.3Cp

Table 4. Crystal formation of formulation

S. No.	Sample No.		Time duration (hrs)		Temperature (°C)	Crystal formulation
1	F_{LD}	24	48	72	4°C	-
2	F_{LD}	24	48	72	RT	-
3	F_{LD}	24	48	72	47°C	-
4	F_{HD}	24	48	72	4°C	-
5	F_{HD}	24	48	72	RT	-
6	F_{HD}	24	48	72	47°C	-

Table 5. Rate of sedimentation volume of formulation

S. No.	Time (min)	Ultimate height (V_u)(ml)	Final volume (V_0)(ml)		Sedimentation volume ratio $F=V_U/V_O$	
			Low dose suspension	High dose suspension	Low dose suspension	High dose suspension
1	30	100	98	99	1.02	1.01
2	60	100	96	98	1.04	1.02
3	90	100	90	93	1.11	1.07
4	120	100	82	85	1.21	1.17
5	150	100	75	78	1.33	1.28
Mean					5.66/5	5.46/5
Total					1.13	1.09

of the herbal suspension. The viscosity and stability of the suspension is increased by carboxymethyl cellulose. Lemon oil is a flavouring agent. Sodium benzoate is a preservative, which is the least harmful preservative and non-toxic[4].

3.2 Chemical Compatibility Test by FTIR

As depicted in Figures 2-10.

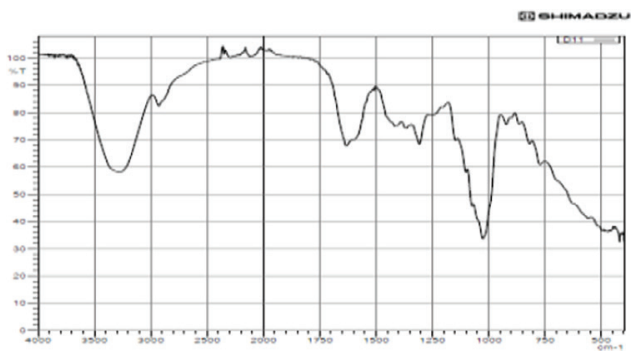

Figure 2. FTIR of *Musa paradisiaca* (D1) combination of *Musa paradisiaca* (D1) and *Punica granatum* (D2)-D1+D2.

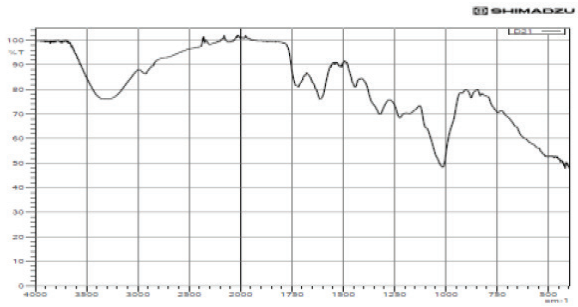

Figure 3. Result of FTIR of *Punica granatum* (D2).

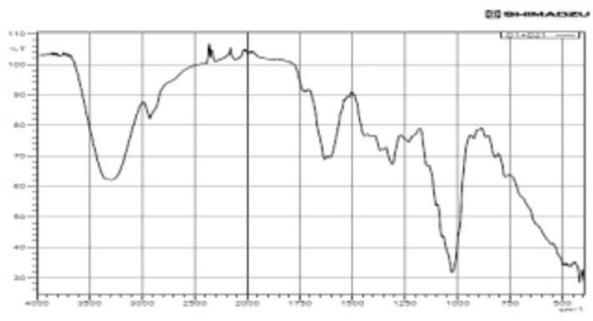

Figure 4. Result of combination of Sodium carboxyl methyl cellulose (A) +*Musa paradisiaca* (D1).

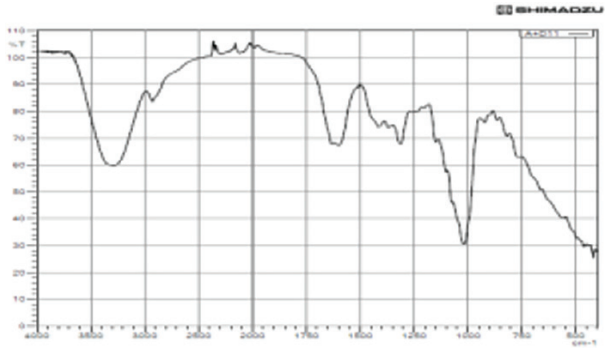

Figure 5. Result of combination of Polysorbate 80 (B) +*Musa paradisiaca* (D1).

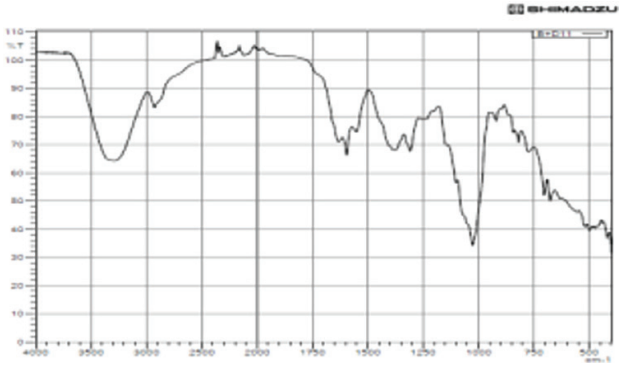

Figure 6. Result of combination of Sodium benzoate (C) +*Musa paradisiaca* (D1).

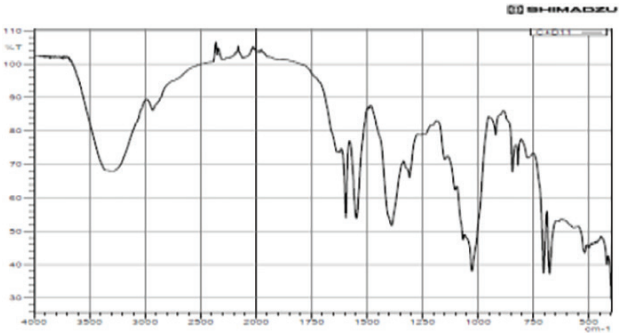

Figure 7. Result of combination of *Punica granatum* (D2) +Sodium carboxyl methyl cellulose (A1).

Chemical compatibility test was performed by FTIR for the suspensions. The objective for performing the chemical compatibility test is to investigate drug- drug interaction and drug excipient interaction[15]. The FTIR spectral analysis measures the change in the frequency and bandwidth of the interacting groups in the spectrum of the pure drug and the excipients. The functional group present in both the plants- *Musa paradisiaca* and *Punica granatum* was alkane, aldehyde, nitrites, ketone, alkyne, aromatic and alkene. The evaluation has resulted

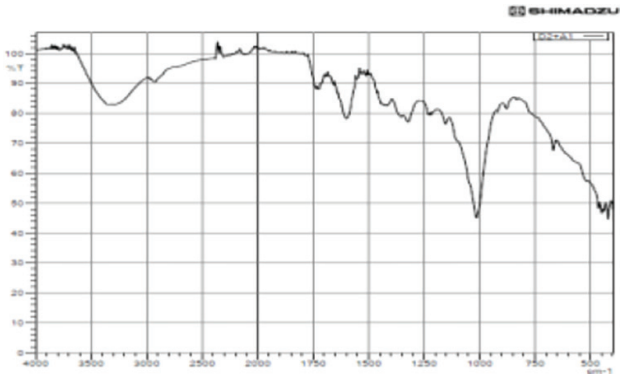

Figure 8. Result of combination of *Punica granatum* (D2) +Polysorbate 80 (B).

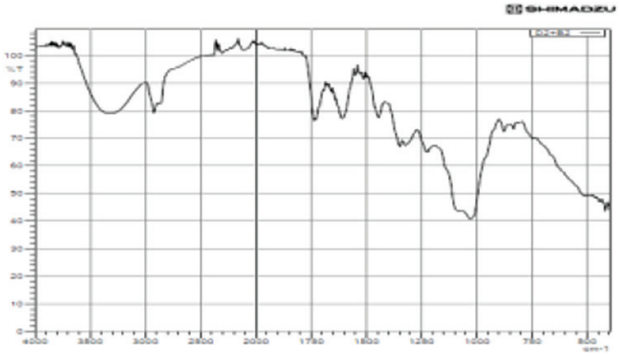

Figure 9. Result of combination of *Punica granatum* (D2)+Sodium benzoate (C).

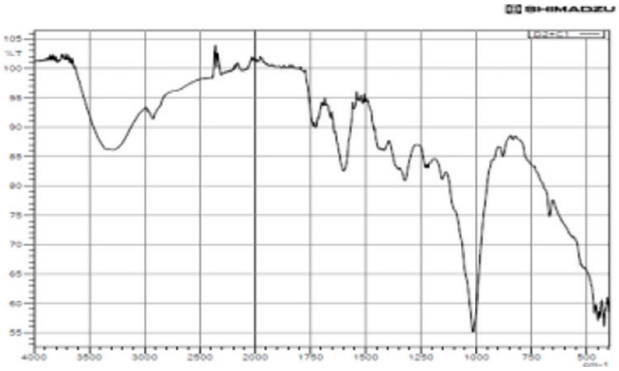

Figure 10. Result of FTIR of *Musa paradisica* (D1).

in no interaction, and no significant difference is noticed in the functional group when combined with both the drugs and drugs with excipients.

3.3 Pharmacological Evaluation

The sterile prostatitis is induced by chemical injection (carrageenan), inflammasomes is activated with elevated NLRP1 (inflammasomes), caspase-1 and

IL-1b level[14]. These result in non-infectious prostatitis The HIN-200 and NLR are the pattern recognition receptors (PPR), which is found in specialized epithelia, immune cells and other tissues[15]. Respond to DAMP's (danger associated molecular patterns) or PAMP's (pathogen associated molecular patterns) forming supramolecular structure known as inflammasomes[16]. These may also combine adaptor molecules known as apoptosis associated speck–like protein (ASC). These structures initiate the cysteine protease caspase-1, which cleaves pro-IL-1b to 1b and pro-IL-18 to IL-18. The mature cytokines are proinflammatory, which triggers inflammatory response[17].

All the values were expressed by Mean+ SEM and were statistically proved by one way ANOVA followed by Dunnetts T test. $p^{*}<0.5$: $P^{**}<0.01$ (Table 6).

Body weight variation is studied as obesity is also a factor which causes prostatitis in men[18]. The body weight variation parameter is assessed because during the prostatitis the animals were subjected to have an increased body weight. The body weight variation of the animals has shown significant weight decrease in the drug-treated and the relative weight increase in the control. The resultant treatment groups like juice, low dose and high dose suspension have maintained the body weight. And, the low dose suspension has shown significant stability in weight when compared to the other groups.

All the values were expressed by Mean+ SEM and were statistically proved by one way ANOVA followed by Dunnetts T test. $p^{*}<0.5$: $P^{**}<0.01$: ns –non significant (Table 7).

In the urine analysis, the control animal has a very low volume of urine excretion. During the day - 3 analysis the group has a very low volume of urine excretion. However, the same group of animals was analyzed on day 7 the intervention group has shown change in the urine volume. Juice treated and low-dose suspension of *Musa granatum* has shown better results during the evaluation.

All the values were expressed by Mean+ SEM and were statistically proved by one-way ANOVA followed by Dunnetts T test. $p^{*}<0.5$: $P^{**}<0.01$ (Table 8).

The prostate gland regulates the path of excretion of urination and motility of the sperms during ejaculation when the prostate gland is subjected to inflammation the path obstructs and causes painful urination and infertility[19]. The weight analysis of the prostate gland confirms that the control animal has a significant increase in prostate gland weight. The evaluation resulted in a decrease in weight in juice and a high dose in the same net weight. The low dose suspension treated group has expressed results in the normal weight.

WBC continues the inflammation occurred in the animal models. The normal WBC counts in rat are 6-17 $\times 103/mm^3$. All the intervention group resulted in the normal range except the disease control has $18.12\pm0.30 \times 103/mm^3$ this further confirms the control group animal in the diseased state.

3.4 Histopathological Evaluation of Prostate Gland

The histological condition in each group was evaluated by morphological changes of prostate acini, lymphocyte

Table 6. Effect of juice and suspension on body weight variation

S. No.	Group	Day -3	Day – 7
1	Normal	147.25±0.98	155.25±1.82
2	Control	154.5±0.49	172.75±1.31
3	Juice	146.5±0.83**	156.25±0.72*
4	Low Dose	146.2±1.27**	154.5±0.89**
5	High Dose	145.7±0.49**	158.25±1.03**

Table 7. Effect of juice and suspension on urine analysis day

		Day 3		Day 7
Group	3 hrs	5 hrs	3 hrs	5 hrs
Normal	0.51±0.01	0.91±0.02	0.64±0.19	1.01±0.01
Control	0.10±0.02	0.18±0.01	0.15±0.14	0.23±0.05
Juice	0.22±0.01*	0.38±0.06**	0.53±0.18*	0.70±0.04**
Low Dose	0.25±0.04ns	0.42±0.08*	0.60±0.17**	0.88±0.08**
High Dose	0.21±0.03**	0.48±0.05**	0.57±0.12**	0.78±0.02**

Table 8. Effect of juice and suspension on prostate gland weight and WBC count

Group	Prostate Gland Weight	WBC Count
Normal	0.27±0.08	7.95±0.13
Control	0.60±0.07	18.12±0.30
Juice	0.38±0.06**	7.12±0.14**
Low Dose	0.30±0.09**	7.78±0.24**
High Dose	0.38±0.012**	7.85±0.11**

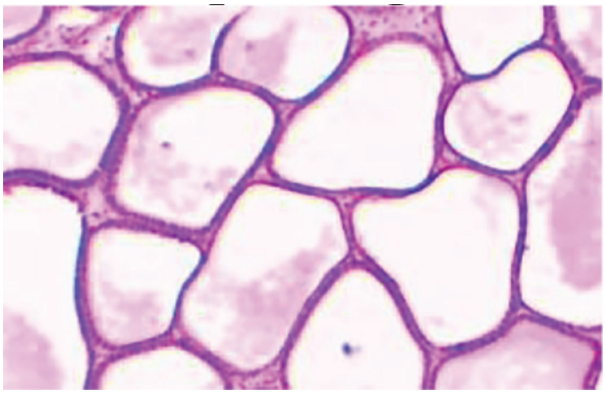

Figure 11. Normal.

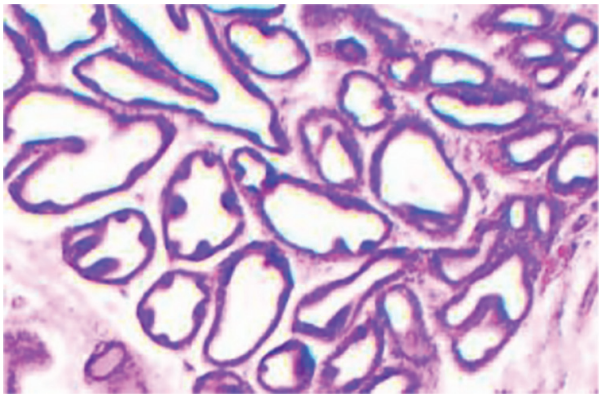

Figure 12: Positive control.

infiltration and interstitial fibrosis. It represented the differentiation between the normal with no signs of inflammation. In Figure 11 Normal control group no signs of cell necrosis, whereas disease control in Figure 12 is identified by focal lymphocyte infiltration in the stroma, hyperproliferation of epithelial cells and also a

reduction in the size of acinar diameter by significant luminal infolding and interstitial proliferation enhancement. This confirms the prostatitis induction on Sprague Dawley rats[20].

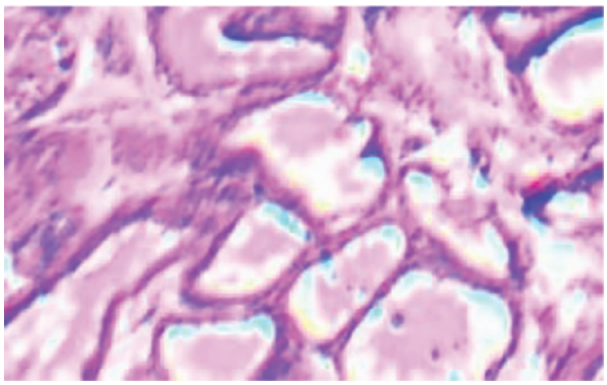

Figure 13. Juice treated.

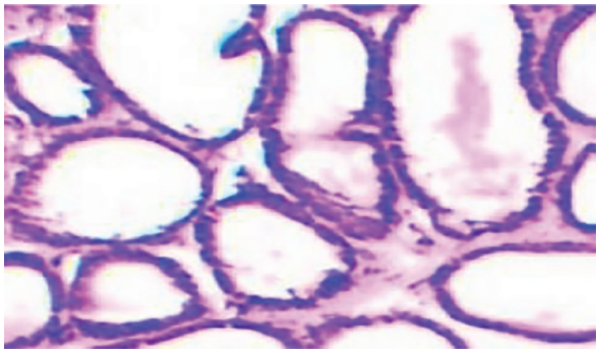

Figure 14. Low-dose suspension.

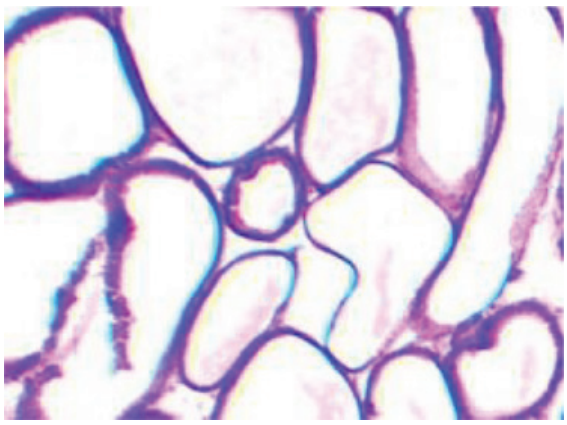

Figure 15. High dose suspension.

In Figure 13, the fresh juice treated group has shown a notable decrease in the luminalinfolding, and in Figure 14 a low dose of *Musa granatum* suspension indicates the increase in the size of acinar diameter and enhanced interstitial proliferation compared to the high dose which is represented in Figure 15. Low-dose suspension has significantly exhibited better results in the histo-morphological changes when compared

to the juice treated, and high dose treated group has denoted the consequential amelioration effect when compared to other groups.

5. Conclusion

The Low dose and high dose of *Musa granatum* suspension have shown effective roles against prostatitis in carrageenan-induced models by reversing the body weight changes, WBC level, Prostate gland weight, urine analysis and histopathological data strengthen the result. Further study has to be designed to perform pharmacokinetic profiling to delineate the therapeutic efficiency.

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