IJPSR (2013), Vol. 4, Issue 1 **(Research Article)**

INTERNATIONAL JOURNAL OF PHARMACEUTICAL SCIENCES AND RESEARCH

Received on 25 September, 2012; received in revised form, 19 October, 2012; accepted, 29 December, 2012

POLYSACCHARIDES FROM ALOE LEAF MUCILAGE AS POTENTIAL IMMUNOLOGICAL-BASED ANTI-FERTILITY AGENTS

Shubhranshu Gupta*, Larissa Pereira, Rohit Dugar and Rajesh Patil

Sinhgad Institute of Pharmaceutical Sciences, Sinhgad Technical Education Society's, Off Pune-Mumbai Expressway, Kusgaon (bk.), Lonavala, Pune-410401, Maharashtra, India

Keywords:

Acemannan, Aloe mucilage, Anti-fertility, GMCSF, Macrophages

Correspondence to Author:

Shubhranshu Gupta

Sinhgad Institute of Pharmaceutical Sciences, Sinhgad Technical Education Society's, Off Pune-Mumbai Expressway, Kusgaon (bk.), Lonavala, Pune-410401, Maharashtra, India

E-mail: guptashubhranshu@yahoo.co.in

QUICK RESPONSE CODE

IJPSR: ICV- 5.07

Website: www.ijpsr.com

ABSTRACT

Mucilaginous exudates from Aloe leaf containing polysaccharides, of which the major proportion has been reported to be composed of acetylated mannose molecules such as Acemannan and other high molecular weight polysaccharides. These polysaccharides, have been reported to elevate the Granulocyte Macrophage Colony Stimulating Factor (GMCSF) ultimately leading to the infiltration of macrophages at respective sites. This immunological property has been exploited to test the efficacy of dried aloe mucilaginous extract containing these high molecular weight polysaccharides for anti-fertility activity. The polysaccharides were extracted by ethanol precipitation method. The suspension of extract in aqueous medium was prepared under aseptic environment to ensure the desired sterility. Characterization of extract was done by spectrophotometry and IR spectroscopy. The results suggest the presence of biologically active polysaccharides. The preliminary finding of the experiment confirms the anti-fertility activity in rats. However, a mild edema was observed in the uterine horns of the test group animals which subsequently reduced in severity upon reducing the dose. The cause of the oedema could be attributed to the excessive endometrial macrophage infiltration and probably due to the IL-1 stimulation at higher doses.

INTRODUCTION: *Aloe vera* is a perennial plant with turgid green leaves joined at the stem in a rosette pattern. The leaves of a mature plant may be more than 25 inches long with saw-like spikes along their margins.

Aloe vera contains two major liquid sources, yellow latex (exudates) and the clear gel (mucilage). The dried exudates of Aloe barbadensis Miller leaves is referred to as aloe.

Aloe mucilage has been reported to contain 98.5-99.2% water and 0.8 to 1.5% solid content primarily comprised of mucilage, fiber, monosaccharide, polysaccharides, proteins, ash, fats, aloin (irritating principle) and resin; of which the polysaccharides have been reported to constitute the major fraction.

The aloe mucilage has been used in folks in treatment of burns, insect stings, bruises, wounds etc. It has been found medically to possess variety of properties like anti-inflammatory, immunostimulant, anti-oxidant, anti-tumor, anti-diabetic, anti-ulcer etc.[1, 2].

Acemannan has been reported to dominate the composition among solid contents [3]. It is a β- linked (1-4) glycosidic polymer containing repeating mannose units with acetyl groups present irregularly.

Acemannan and other high molecular polysaccharides have been reported to possess a significant ability to stimulate the Granulocyte Macrophage colony stimulating factor (GMCSF), which result in enhanced infiltration of macrophages. Besides this, these carbohydrates also stimulate production of IL-1, which might account for pyrogenic effect and inflammation. These polysaccharides also lead to lymphocyte activation [4, 5].

These high molecular weight sugars have been reported to activate the macrophages. This activation leads to an increased production of GMCSF by uterine macrophages, leading to an increased production of macrophages by the stem cells. This eventually converts the small population of uterine macrophages into a large localized macrophage population. The details of the mechanism could be referred in **Fig. 1**.

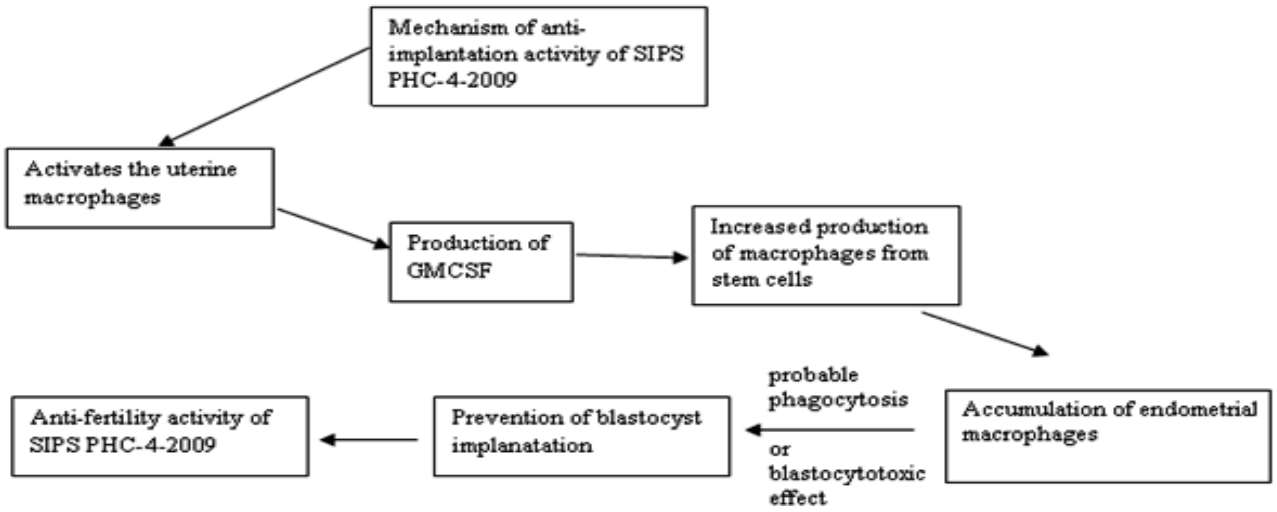

FIGURE 1: MOLECULAR AND IMMUNOLOGICAL CASCADE TRIGGERED BY SIPS PHC-4-2009 LEADING TO ANTI-FERTILITY ACTIVITY

(SIPS PHC-4-2009: Aqueous suspension of the extract, GMCSF: Granulocyte Macrophage Colony Stimulating Factor)

The current study is based on the investigation of anti-implantation activity of the extract SIPS PHC-4-2009. The primary purpose of this experiment was to investigate the role of these high molecular weight polysaccharides as effective contraceptive agents.

The gist of the hypothesis revolves around the immunostimulating nature of the polysaccharides which defines its immunological role in contraception by preventing implantation of the zygote. This phenomenon has been observed to occur in women on steroidal contraceptives wherein the endometrial wall has a highly increased infiltration of Macrophages and Lymphocytes [5]. Moreover, research has strongly emphasized the role of Endometrial Macrophages in phagocytosing the newly introduced sperms in the uterus [6]. These data were sufficient to allocate some inhibitory roles to the endometrial macrophages in preventing conception.

Contraception achieved this way would be beneficial from many aspects. Firstly, it would use body's own mechanism rather than bringing about the desired pharmacology by changing the hormonal status, which is associated with a lot of systemic toxicity. This would also limit the side-effects to the local area rather than ushering toxicity throughout the body.

Secondly, being a carbohydrate, it would possess a short half-life (and would be eliminated rapidly by the body easily) in contrast to the long half lives of the steroidal contraceptives.

MATERIAL AND METHODS: Marketed dried mucilage powder from Aloe was purchased from the local market in Pune, India in 2009. The acemannan was prepared by ethanol precipitation method [7] under aseptic conditions, which also removed Aloin (possessing irritant activity). The aqueous suspension of the extract (SIPS PHC-4-2009) was then filtered through 0.45 µ membrane filter to sterilize the formulation. The step of lyophilisation was excluded and the extract was used immediately after passing through the membrane filter. The Phytochemical screening tests were done on the extract for carbohydrates, polysaccharides, anthra-quinones and proteins [8].

The presence of active polysaccharides were ensured by spectrophotometric determination at 540 nm by Dye complexing technique. Congo red dye was chosen as the complexing dye and spectrophotometry was done at 540 nm wavelength [9]. The FTIR was performed in Jasco FT/IR-4100.

Male and female albino Wistar rats were received as free gifts from National Toxicology Center, Pune, India and were acclimatized in disease-free animal facility for 2 weeks before use. Animals were given healthy food and clean water as well as housed under standard 12h light: 12 h dark cycle at normal room temperature of 25±3 °C and humidity of 65 ± 10%. Handling of rats was done according to the guidelines for care and use of laboratory animals. The Anti-fertility activity [10] was determined following the method of Khanna and Chowdhury. Male and female albino Wistar rats of proven fertility (150-200 g) were maintained under controlled standard animal house conditions with *ad libitum* access to food and water.

Vaginal smears from each rat were monitored daily. Female rats of proestrus phase were kept with male rats in the ratio of 2:1. The females were examined the following morning for evidence of copulation. The animals which showed thick plugs of spermatozoa in vaginal smears were separated from the male partner and divided into two groups: control (n=5) and test.

The test group in turn was further sub-divided into 4 sub-groups (n= 5 each) with group A, B, C and D (**Figure 3**). The day, when spermatozoa were detected in the vaginal smear was considered as day 1 of pregnancy. Sterile water (control) and sterile extract (Test) were cautiously administered intravaginally in all groups, except B on 0^{th}, 2^{nd} and 4^{th} day. Group B rats were dosed only on the 0^{th} and 4^{th} day.

The females were sacrificed on the 8^{th} day using diethyl ether anesthesia and the uterine horns were observed for the presence of implants.

The Control group rats received sterile distilled water while the treated group received a dose of 4, 4, 3 and 2 mg aqueous suspension of the extract in Groups A, B, C and D respectively. Total sterility of distilled water was ensured by UV-sterilization for 30 minutes, just before the administration.

Statistical analysis was carried out using t-test. $P \leq 0.05$ was considered statistically significant.

RESULTS AND DISCUSSION: By phytochemical screening, it was observed that the extract contained carbohydrates and polysaccharides, but not Anthraquinones and proteins. In the study of evaluation of the extract by IR spectroscopy (**Fig. 2**) of SIPS PHC-4-2009, we noticed that the wave numbers characteristic of O-C stretching, C-H stretching, O-H stretching, C-H bending and C-C stretching were present, further indicating the presence of poly saccharides. The UV spectroscopic results (**Table 1**) speak of similar conclusions.

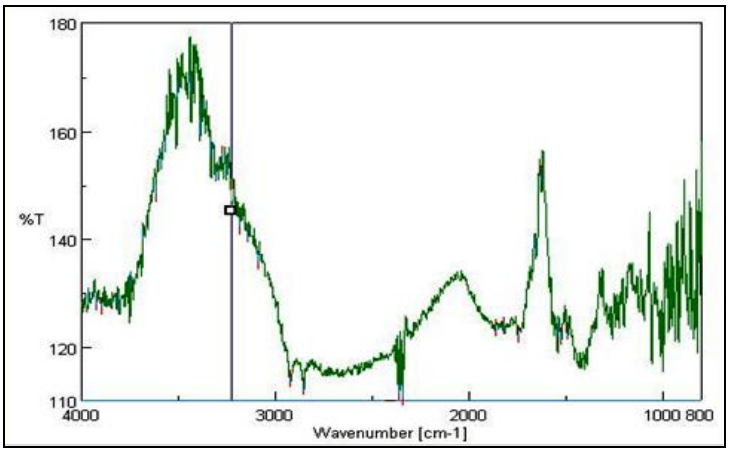

FIGURE 2: FTIR OF SIPS PHC-4-2009. The peaks were found at wave numbers characteristic of O-C stretching, C-H stretching, O-H stretching, C-H bending and C-C stretching.

TABLE 1: THE PRESENCE OF ACEMANNAN AND HIGH MOLECULAR WEIGHT POLYSACCHARIDES BY SPECTROPHOTOMETRY AT 540 NM

Sr. No.	Concentration of MMP (%w/v)	Absorbance	Concentration of EXT (%w/v)	Absorbance
1	5.4	0.5734	5.0	0.9639
2	5.8	0.6086	5.4	1.0353
3	6.2	0.6213	5.8	1.1067
4	6.6	0.6696	6.2	1.1781
5	7.0	0.6756	6.6	1.2495
6	7.4	0.7402	7.0	1.2765
7	7.8	0.7426	7.4	1.3078

MMP: crude marketed mucilage powder, EXT: extract.

TABLE 2: EFFECT OF TEST AND CONTROL GROUPS ON UTERUS OF FEMALE ALBINO WISTAR RATS

Nature of the group	Group	Dose of the extract (mg/rat)	Observation
Test Groups	A	4	Uterine horns with oedema, but no implants detected
	B	4	Lesser number of implants
	C	3	Uterine horns with lesser oedema and no implants
	D	2	Uterine horns with lesser oedema and no implants
Control Group	E	0	10-14 implants

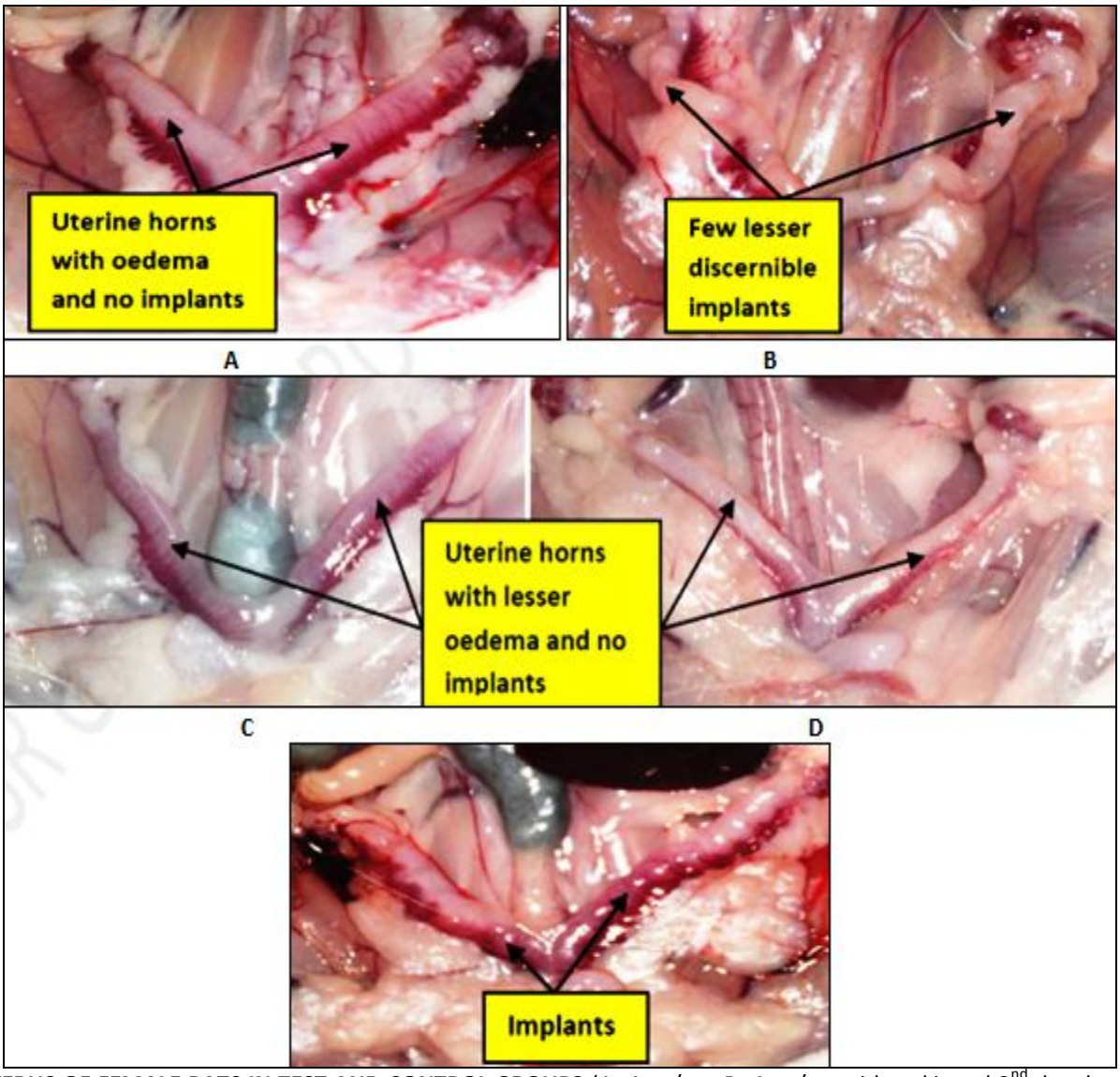

FIGURE 2: UTERUS OF FEMALE RATS IN TEST AND CONTROL GROUPS (A- 4mg/rat, B- 4mg/rat with a skipped 2nd day dose, C- 3mg/rat, D- 2mg/rat and E- Control group). In group B, dose on the 2nd day was skipped; while in group E, only sterile water (without the extract) was instilled.

From Figure 3 and Table 2, it could be observed that the implants were absent in the uterine horns of females of the test group, whereas the animals in control group showed the presence of varying number of implants in both the uterine horns. This shows that the extract possesses a significant anti-fertility activity. However, mild edema was observed in the uterine horns of the test group animals, which subsequently reduced in severity upon reducing the dose. This could be attributed to the excessive endometrial macrophage infiltration and probably due to the IL-1 stimulation at higher doses.

The water retention was, however, observed to reduce at lower doses establishing the fact, that a dose which is both pharmacologically effective as well as yielding least edema, are yet to be ascertained by Dose-Finding experiments.

This research has put more experimental emphasis on the role of myeloid cells in contraception. Simultaneously, the current work has brought a carbohydrate into the limelight for the first time, which could act as a contraceptive through an immunological mechanism. This also explains the impeding effect of any agent, having an immuno-stimulant effect, on the zygote when pregnancy is desired.

Although, this research has proven the potential of immunostimulants to prevent conception, a lot of research is still required to be done to find out the actual carbohydrate in the pool of polysaccharides, experimentally investigate the mechanism of anti-implantation activity and the complete toxicology profile of the active agent.

ACKNOWLEDGEMENT: The authors are thankful to Dr. SR Naik and Mr. Vishnu Thakare for their guidance. The authors would also like to thank Board of College and University Development, University of Pune for their funding and National Toxicology Centre, Pune for issuing rats.

REFERENCES:

1. Ozsoy N, Candoken E and Akev N: Implication for degenerative disorders . Oxidative Medicine and Cellular Longevity 2009; 2:99-106.
2. Reuter J, Jocher A, Stump J, Grossjohann B, Franke G and Schempp CM: Investigation of the anti-inflammatory potential of Aloe vera gel (97.5%) in the ultraviolet erythema test. Skin Pharmacology and Physiology 2008; 21:106-110.
3. Pugh N, Ross SA, ElSohly MA and Pasco DS: Characterization of Aloeride, a new high-molecular-weight polysaccharide from *Aloe vera* with potent immunostimulatory activity. Journal of agricultural and food chemistry 2001; 49:1030-1034.
4. Lee CK: Immunomodulatory activity. New Perspectives on Aloe 2006; 4:155-167.
5. Egger SF, Brown GS, Kelsey LS, Yates KM, Rosenberg LJ and Talmadge JE: Hematopoietic augmentation by a β-(1,4)-linked mannan. Cancer Immunology, Immunotherapy 1996; 43:195-205.
6. Muscato JJ, Haney AF and Weinberg JB: Sperm phagocytosis by human peritoneal macrophages: a possible cause of infertility in endometriosis. American Journal of Obstetrics and Gynecology 1982; 144:503-510.
7. Nema J, Shrivastava SK and Mitra NG: Physicochemical study of acemannan polysaccharide in *Aloe species* under the influence of soil reaction (pH) and moisture application. African Journal of Pure and Applied Chemistry 2012; 6:132-136.
8. Usman H, Abdulrahman FL and Usman A: Qualitative Phytochemical Screening and *In Vitro* Antimicrobial Effects of Methanol Stem Bark Extract of *Ficus Thonningii* (Moraceae). African Journal of Traditional, Complementary and Alternative Medicine 2009; 6:289-295.
9. Alexis AR, Eberendu AN and McAalley BH: Colorimetric assay for bioactive polysaccharide. United States Patent 1996; Patent number 5512488.
10. Koneri R, Saraswati CD, Balaraman R and Ajeesha EA: Antiimplantation activity of the ethanolic root extract of *Momordica Cymbalaria* Fenzl in rats. Indian Journal of Pharmacology 2009; 39: 90-96

How to cite this article:
Gupta S, Pereira L, Dugar R and Patil R: Polysaccharides from Aloe leaf Mucilage as Potential Immunological-based Anti-fertility agents. *Int J Pharm Sci Res*. 2013; 4(1); 440-444.