Journal of Chemical Health Risks

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JCHR (2024) 14(5), 1973-1986 | ISSN:2251-6727

Design, Optimization and Characterization of *Achyranthes Aspera* Extract Loaded Nanogel for Effective Treatment of Acne and Aging

Shivani Samaiya*[1], Dr. Virendra Kumar Sharma[2]

[1]Research Scholar, [2]HOD Pharmaceutics, School of Pharmacy, LNCT University, Bhopal (M.P.), India

***Correspondence to Author**

Shivani Samaiya
Research Scholar Department of Pharmaceutics, School of Pharmacy, LNCT University, Bhopal (M.P.), India, 462023

(Received: 11 June 2024 Revised: 16 July 2024 Accepted: 10 August 2024)

KEYWORDS

Achyranthes aspera, Hyaluronic acid, nanogel, extracts, in-vivo, iv-vitro, PVA

ABSTRACT:

The Achyranthes aspera extract loaded nanogel was successfully prepared optimized and in-vitro and in-vivo evaluated to enhance the potential of herbal dug and stabilized the formulation. The crude extract was screened for the presence or absence of secondary metabolites. Physiochemical analysis of dry powders of Achyranthes aspera leaves was performed. Hyaluronic acid and polyvinyl alcohol were water soluble substances but dissolved slowly so the warm water was used to solubilize the polymers. Various percentages of polymers were mixed. Free radical scavenging activity of samples was measured using DPPH and The ABTS radical scavenging methods. The results exposed that the particle size was 302 ± 12 nm, Gelling time 6.3 ± 0.26 min and entrapment efficiency found (89.92 ± 0.92) % for AA-4 batch. The AA-4 was selected as optimized batch for the further evaluation. The investigation results of antioxidant activity of Achyranthes aspera extracts and extract loaded nanogels were performed as DPPH and ABTS radical scavenging activity. It was concluded that the Achyranthes aspera extracts and nanogel AA-4 possessed potential to scavenge free radicals it will helps to reduces aging because the main cause of aging is free radicals. It was also concluded that the developed nanogel provides unique mechanism to deliver drug effectively at inflamed ear of mice when applied topically. Both delivery and biological activity are significantly enhanced, which was found not to be pro-inflammatory. In a therapeutic context, this Achyranthes aspera loaded nanogel and A. aspera nanogel delivery system would potentially useful for the topical delivery of a drugs.

INTRODUCTION

Plants and natural products were used by humankind over the years as food and medicines to cure and prevent diseases[1]. Nanogels can be defined as a type of systemic drug delivery carrier, are hydrogels with 3D tunable porous structure and a particle size in the submicrometer range, from 20 to 250 nm, formed by physically or chemically crosslinked polymeric chains with a high capacity to absorb water without actually dissolving into an aqueous medium[2]. Acne is an infectious disease and characterized by different areas of scaly red skin seborrhea, pinheads blackheads and whiteheads, large papules and sometimes scarring[3]. Skin aging is a complex biological route influenced by combination of intrinsic (genetics, cellular metabolism, hormone and metabolic processes) and extrinsic (chronic light exposure, pollution, ionizing radiation, chemicals, toxins) factors[4]. These factors lead together to cumulative structural and physiological alterations and progressive changes in each skin layer as well as changes in skin appearance, especially, on tsun-exposed skin areas[5]. *Achyranthes aspera* Linn (*Amaranthaceae*)

is an erect stiff medicinal plant available as weed in whole India[6]. Whole plant Juice is traditionally used for treatment of the dysentery, boils, diarrhea, haemorrhoids, rhematic problems, Skin problems[7,8].

The folklore plants and extracts has been widely investigated, and the phytochemical investigations from both hemispheres led to the isolation of a number of physiologically active compounds viz., tannins, alkaloids, iridoids, and carbohydrates[9]. The basic line of mechanism to treat *acne vulgaris* involves series of actions starts from inflammation, wound management and chelation. Inflammation followed by generation of wound is the main cause of bacterial formation[10]. The crude drugs selected for the proposed study contains glycosides, flavonoids, alkaloids and many more phytoconstituents which prominently used in the line of treatment of *acne vulgaris*[11]. The herbal medicines have a proven long history of treating dermatological conditions and have been reported to be effective and safe for acne and aging[12]. The literature revealed that there are many other formulations are available in the market like cream and ointment which may be useful for treatment of acne but was no report or data present on the nanogel formulation of these plants[13]. Nanogels are great importance in palliative products for curing pain & inflammation associated with arthritis, alkalosis, skin eruptions, eczema, dermatitis, rash etc. Hence it was decided to formulate an effective nanogel of extracts[14]. The nanogel promotes the better absorption and fosters the better mechanism than any other type of delivery system[15]. Therefore attempt was made to evaluate the herbal anti acne formulation followed by biological screening. On the basis of this traditional claim, of these selected oriental plants alcoholic extracts were used for the desired action.

MATERIALS AND METHODS

Preliminary Work: The Leaves of plant *Achyranthes aspera* was collected locally from Bhopal, Madhya Pradesh, India. Powdered material of *Achyranthes aspera* extracted using ethanol by Soxhlet extraction method.

Quantitative studies of bioactive constituents: The plant extract was subjected for total alkaloids content, total phenol content and total flavonoids content by using standard procedure.

Optimization of formulation (3^2factorial design) for plant extracts containing nanogel:

A 3^2 randomized full factorial design was utilized in the present study. Two factors were evaluated, each at three levels, to further carry out nine experimental trials as all possible combinations. Hyaluronic acid (HA) and polyvinyl alcohol (PVA) were main polymer in nanogel preparation hence, concentration of Hyaluronic acid and PVA was selected as independent variables. Particle size was important evaluation parameter of nano dosage formulation; gelling time was the time of polymer mixed with chemical cross linker conversation to gel, Drug entrapment efficiency means drug entrapped in nano formulation.

Method of Preparation of plant extracts containing nanogels:

Achyranthes aspera extract containing nanogel was prepared by chemical cross linker gel method. In the process 100 mg Herbal extract was dissolved in 10 ml distilled water. Hyaluronic acid (0.5%w/v, 1%w/v, 1.5%w/v) was dissolved in distilled water at room temperature for 1 hr on magnetic stirrer and PVA (Different concentration like 2.5% w/v, 5%w/v, 7.5%w/v) were dissolved in distilled water at 50 ^{O}C on magnetic stirrer for 12 hour (1 hour for dissolve and further 11hr to make homogeneous solution). 1ml of DMF solution was added in 5ml of Hyaluronic acid solution and 3ml PVA Solution was mixed and the mixed solution was stirred at room temperature for 2 hrs. PVA was cross linked in the presence of HA, using 1ml Glutaraldehyde (GA) (25 %w/v) and 0.2 ml Hydrochloric acid (HCl) (6%w/v) as a crosslinking agent and catalyst.

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JCHR (2024) 14(5), 1973-1986 | ISSN:2251-6727

Table No. 1: The 3^2 factorial design for optimization of Polymer Concentration with *Achyranthes aspera* Extract (AAE)

Batch No.	HA (%W/V)	PVA (%W/V)	AAE extract (mg)	Glutaraldehyde (25%V/V)	HCl (6% V/V) ml
AA-1	0.5	2.5	100	1	0.2
AA-2	0.5	5.0	100	1	0.2
AA-3	0.5	7.5	100	1	0.2
AA-4	1.0	2.5	100	1	0.2
AA-5	1.0	5.0	100	1	0.2
AA-6	1.0	7.5	100	1	0.2
AA-7	1.5	2.5	100	1	0.2
AA-8	1.5	5.0	100	1	0.2
AA-9	1.5	7.5	100	1	0.2

Characterization of Herbal extract containing nanogel: Prepared nanogels were characterized for Appearance, Viscosity, pH, Particle size measurement and zeta potential, Percent drug entrapment efficiency, Free drug measurement, Entrapped drug measurement, Gelling Time and *In-vitro* drug release study using Franz diffusion cell.

Antioxidant activity of *Achyranthes aspera* Extract (AAE): Free radical scavenging activity of samples was measured using 2,2-diphenyl-1-picrylhydrazyl (DPPH) and ABTS radical (2,2'-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid)) scavenging methods.

***In-vitro* antimicrobial evaluation of prepared optimized formulations:** *In-vitro* antibacterial activities of *Achyranthes aspera* was determined by standard Disc diffusion assay method.

***In-vivo* anti-inflammatory activity of prepared optimized formulations**

Animals care and Handling: The animal experimental protocol was approved by the Institutional Animals Ethical Committee (IAEC) of Sagar Institute of Research & Technology-Pharmacy, Sanjeev Agrawal Global Educational University, Bhopal, (M.P). Approval Number, Ref/15/IAEC/Pharmacy 2024 Dated: 20/04/2024. Male & female Wistar albino rats (140-170g) were provided by National Institute of Nutrition ICMR, Jamia Osmania, Hyderabad, India. The animals were housed in standard conditions of temperature (25 ± 2 ^{O}C) and 12:12 h light-dark cycle. The rats were fed with commercial diet and water *ad Libitum.*

***In-vivo* anti-inflammatory activities using Croton Oil induced ear edema model:** Healthy animal will be selected, randomized based are body weights and divided into 8 different groups containing of 6 animals each. Before starting the experiment animals will be housed for 14 days in controlled environment. Ear edema was induced by 20 µl of a fresh solution of 2.5% croton oil were topically applied on the inner surface of the right ear, while in the left ear an equal volume of acetone (vehicle) was applied as control.

Table No. 2: Experimental design and group distribution of animal

Group	Drug to be administered	Dose & Route of Administration	Volume of Drug to be administered	No. of animals
Group-I	2.5% Croton Oil	20 µl	20 µl	6
Group-II	Dexamethasone	0.02 Ear Skin surface	20 µl	6
Group-III	*Achyranthes Aspera* Extract	1.0 mg/Ear Skin surface	20 µl	6
Group-IV	*Achyranthes Aspera* nanogels (AA-4)	1.0 mg/Ear Skin surface	20 µl	6

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JCHR (2024) 14(5), 1973-1986 | ISSN:2251-6727

Estimation of thiobarbituric acid reactive substances (TBARs)[16]: Oxidative damage to lipids (lipid peroxidation) was determined by TBARs formation assay during acid-heating reaction, as previously described in literature with a few modifications**.** The results were expressed as µmol of MDA equivalents per ear.

Determination of thiol groups[17]: Oxidative damage to proteins (reduced glutathione level) was determined by sulfhydryl (SH) group formation using 5,5′-dithiobis(2-nitrobenzoic acid) (DTNB) method, as previously described in literature with a few modifications**.** The results were expressed as µmol of TNB equivalents per ear.

Histopathological analysis[18]: Ear biopsies were fixed in 10% buffered formaldehyde solution, dehydrated and embedded in paraffin. Sections of 5 µm of thickness were obtained for haematoxylin-eosin staining and examined by light microscopy. The distance between the epithelium of inner and outer face of the ears was measured in micrometers, as index of edema.

RESULTS AND DISCUSSION

Preliminary Work

Morphological characteristics of plants: Plants were collected locally from from Bhopal, and authenticated by Department of Botany Saifia Science College, Bhopal. Dried plant leaves were powder to obtain a coarse powder and sieve. Morphological characteristic of *Achyranthes aspera* was green in color having characteristic odor with rough surface textures. Leaves were 4-8 cm long and 1.5-4.0 cm broad in size.

Physiochemical analysis of dry powders: Physiochemical analysis of dry powders of *Achyranthes aspera* was performed using parameters e.g. Loss on drying (9.4), Total ash value (8.3), Acid insoluble ash value (3.4), Water soluble ash value (1.7) and Foaming index (4) ml.

***A. aspera* extract:** *A.A.* extract was dark green semisolid having 11.4% yields. Phytochemical screening of extracts reveals that Alkaloids, Glycosides and Flavonoids were present.

Estimation of total Alkaloids, Phenol and Flavonoids content in *Achyranthes aspera* extract (AAE): Total Alkaloids Content (0.869 mg/100mg), Total Phenol Content (0.574 mg/100mg) and Total Flavonoids Content (0.365 mg/100mg).

Drug and Excipients Compatibility Study by FT-IR Spectra:

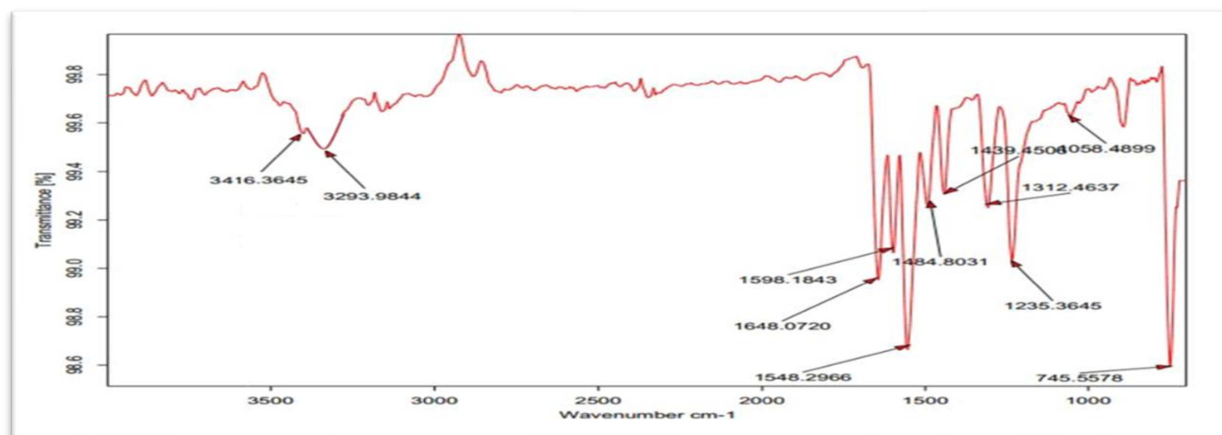

Figure 1: FT-IR Spectra of *Achyranthes aspera* Extract

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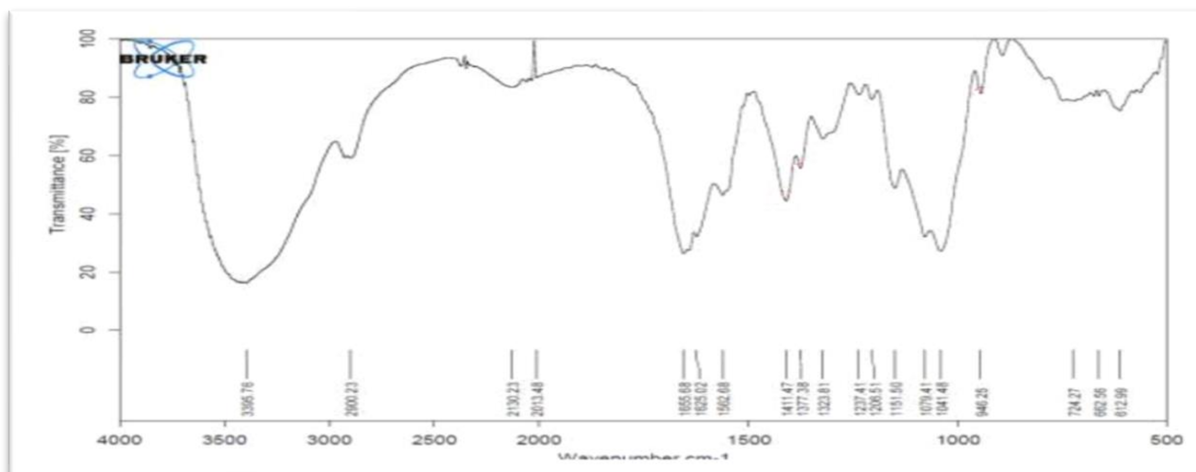

Figure 2: FT-IR Spectra of Hyaluronic Acid

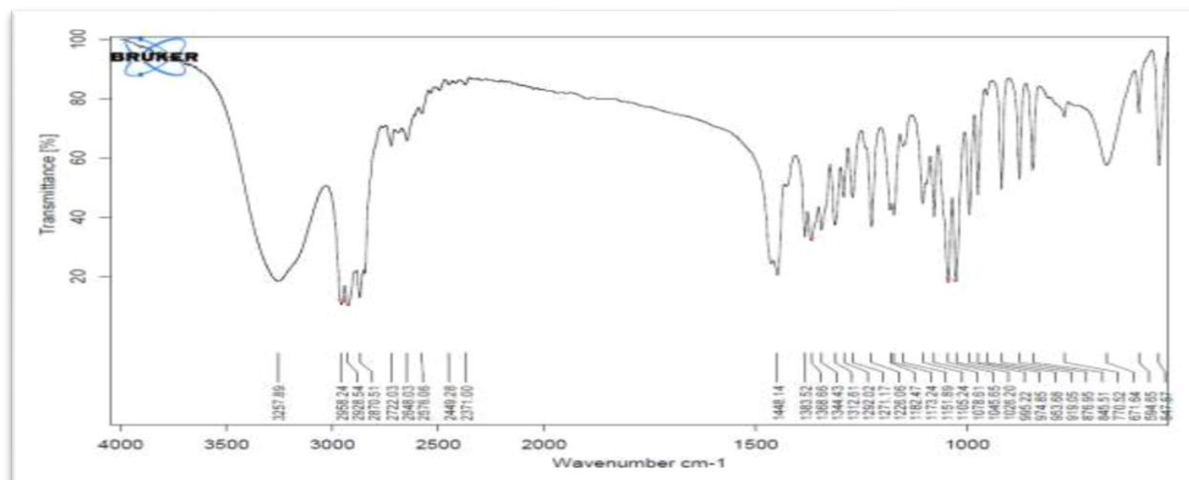

Figure 3: FT-IR Spectra of Polyvinyl alcohol

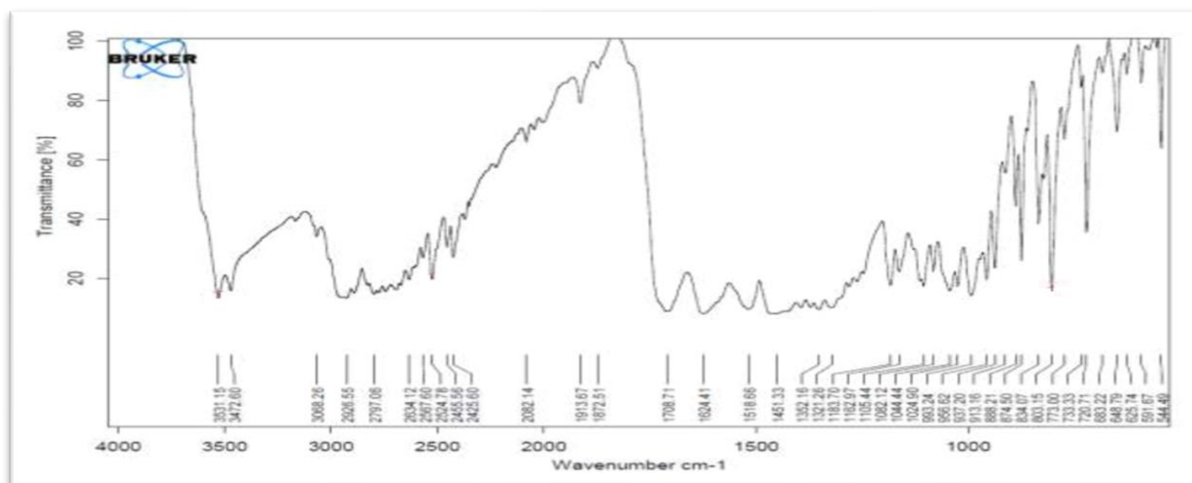

Figure 4: FT-IR Spectra of HA+PVA+ *Achyranthes aspera* Extracts

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Based on results of above FT-IR Spectras it was concluded that drug alone, mixtures with extracts which are compatible with each other. It was concluded that after product development no degradation was found in drug from formulation.

Formulation of herbal extract loaded Nanogels

Selection of polymers by hit and trial method: Polymers were selected by hit and trial method among different available polymers e.g. polyvinyl alcohol (PVA), Hyaluronic acid (HA), dextran (DX) and Chitosan (CS) using different concentrations. Hyaluronic acid was used as primary polymer with reported concentration e.g. 1.0%, 2.0%, 3.0% while PVA, dextran and chitosan were tried as secondary polymers with concentrations of 2.5%, 5% and 7.5%. Chemical cross linker method was used to prepare Nanogels. In this method, as chemical cross linker Glutaraldehyde (GA) (25% w/v) was used and a catalyst HCl (6% v/v) was used for pH adjusting agent. Different proportions of polyvinyl alcohol (PVA), dextran (DX) and Chitosan (CS) was mixed with Hyaluronic acid (HA) to get the PVA-HA, dextran-HA, chitosan-HA solution. Chemical cross linker and HCl were added in HAPVA solution. In 10 ml of HAPVA solution, around 1.2 ml of Glutaradehyde and HCl were added with polymer to crosslinker molar ratio maintained by 0.85.

Trial-1: Hyaluronic acid was water soluble polymer while Chitosan was soluble in dilute aqueous acidic solution (pH <6.5) (0.1 M HCl). Various percentages of polymers were mixed as described in above table. The observations of trial batches from F-1 to F-9 were indicated that, all batches found to be clear, thick solution but no gel formation found. Thus, it was concluded that the nanogel cannot be formulate with combination of hyaluronic acid and Chitosan.

Trial-2: Both polymers Hyaluronic acid and Dextran were water soluble. Various percentages of polymers were mixed as described in above table. Nine trial batches were prepared. The observations of trial batches from F-10 to F-18 were indicated that, all batches found to be clear, thick to hard solution. It revealed that, crosslinker and HCl were not reacted with polymers. Thus, it was concluded that the nanogel cannot be formulate with combination of hyaluronic acid and Dextran.

Trial-3: Although, Hyaluronic acid and polyvinyl alcohol were water soluble substance but dissolve slowly, so warm water was used to solubilize the polymers. Various percentages of polymers were mixed as described in above table. Nine trial batches were prepared. Trial batches F-19, F-20 and F-21 were optimum gels. Rest of prepared trial batches showed thick solution with lump, as unstable gel.

Table No. 3: Trial for selection of Polymers of Hyaluronic acid and polyvinyl Alcohol

Formulation	Polymers		Crossliker	Catalyst	Observation
	HA (%)wt	PVA (%)wt	GA (25 % w/v) in ml	HCl (6% v/v) in ml	
F-19	**1 %**	**2.5 %**	**1 ml**	**0.2 ml**	**Optimum gel formulation**
F-20	**1 %**	**5 %**	**1 ml**	**0.2 ml**	**Optimum gel formulation**
F-21	**1 %**	**7.5 %**	**1 ml**	**0.2 ml**	**Optimum gel formulation**
F-22	2 %	2.5 %	1 ml	0.2 ml	Thick solution with lump, as a unstable gel
F-23	2 %	5 %	1 ml	0.2 ml	Thick solution with lump, as a unstable gel
F-24	2 %	7.5 %	1 ml	0.2 ml	Thick solution with lump, as a unstable gel
F-25	3 %	2.5 %	1 ml	0.2 ml	Lump formed
F-26	3 %	5 %	1 ml	0.2 ml	Lump formed
F-27	3 %	7.5 %	1 ml	0.2 ml	Lump formed

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JCHR (2024) 14(5), 1973-1986 | ISSN:2251-6727

Based on observations of preliminary trials for selection of polymers, it was concluded that Hyaluronic acid - Polyvinyl alcohol (HA-PVA) was possessed optimum polymers for further optimization. So, HA-PVA combination within (0.5 to 1.5% w/w HA and 2.5 to 7.5% w/w PVA) were taken for further optimization.

Optimization of Formulation of *Achyranthes aspera* Extracts containing Nanogel:

Table No. 4: Optimization of formulation variable using 3^2 Factorial Design of *Achyranthes aspera* extract loaded Nanogels

Batch No.	HA (%W/V)	PVA (%W/V)	Gelling time (min)	Entrapment Efficiency (%)	Particle Size (nm)
AA-1	0.5	2.5	6.4 ± 0.62	65.63 ± 0.82	402 ± 12
AA-2	0.5	5.0	7.2 ± 0.21	69.72 ± 1.72	412 ± 21
AA-3	0.5	7.5	7.4 ± 0.55	76.62 ± 1.06	461 ± 15
AA-4	**1.0**	**2.5**	**6.3 ± 0.26**	**89.92 ± 0.92**	**302 ± 12**
AA-5	1.0	5.0	6.8 ± 0.16	86.42 ± 1.21	332 ± 18
AA-6	1.0	7.5	6.8 ± 0.35	83.47 ± 0.75	371 ± 10
AA-7	1.5	2.5	7.4 ± 0.06	80.74 ± 0.72	390 ± 14
AA-8	1.5	5.0	7.8 ± 0.17	75.16 ± 0.31	416 ± 13
AA-9	1.5	7.5	8.2 ± 0.31	72.82 ± 0.18	471 ± 25

(n=3, Mean ± SD)

The observation of the results obtained from the above table of optimization using 3^2 factorial designs it was clear that the formulation AA-4 has the minimum particle size and maximum drug entrapment. As the results exposed that the particle size was 302 ± 12 nm, Gelling time 6.3 ± 0.26 min and entrapment efficiency found (89.92 ± 0.92) % for AA-4 batch. The AA-4 was selected as optimized batch for the further evaluation.

Characterization of *Achyranthes aspera* extract (AAE) loaded nanogels

Organoleptic properties of prepared *Achyranthes aspera* extract loaded Nanogel was observed. All prepared nanogels were green transparent, homogeneous, clear and having characteristic odor.

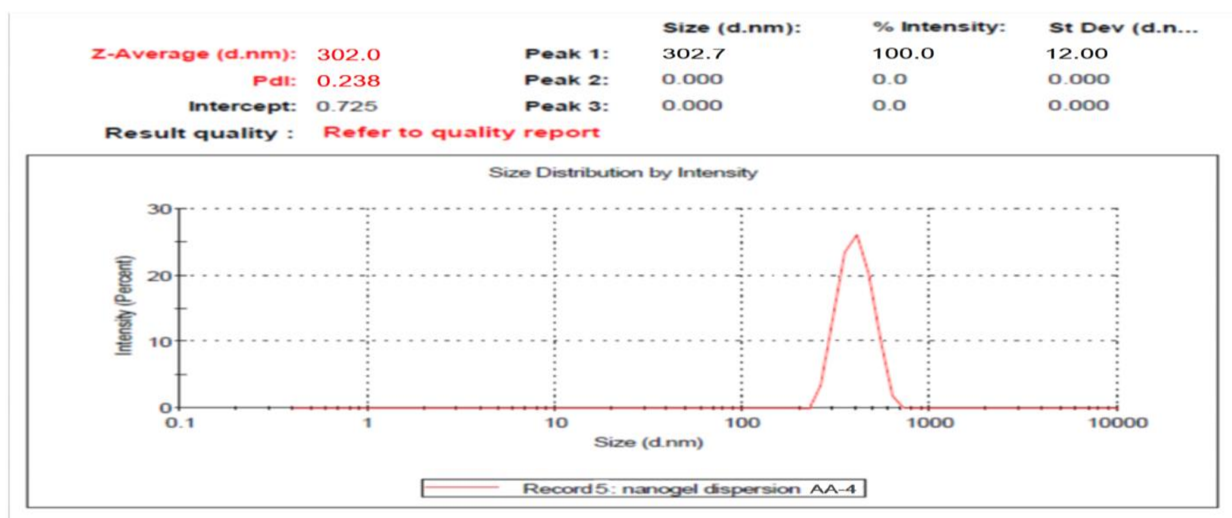

Figure 5: Particle size of optimized batch AA-4

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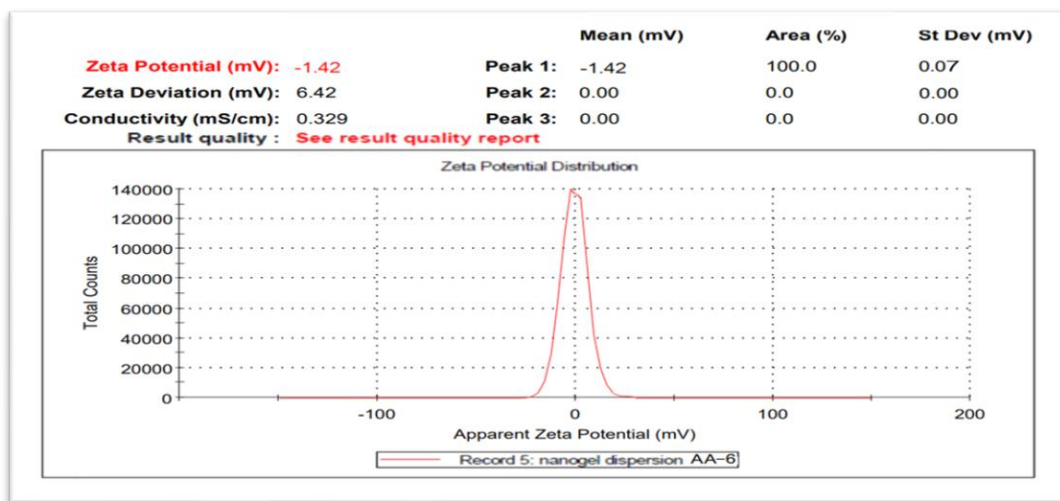

Figure 6: Zeta potential of optimized batch AA-4

Table No. 5: Characterstic properties of *Achyranthes aspera* extract (AAE) loaded nanogel

S. No.	Batches	Viscosity (cp)	pH	Zeta potential	PDI Mean ± SD
1.	AA-1	684	6.7	-1.34 ± 0.11	0.193 ± 0.034
2	AA-2	701	6.8	-1.38 ± 0.12	0.212 ± 0.037
3.	AA-3	742	7.0	-1.42 ± 0.11	0.231 ± 0.026
4.	**AA-4**	**760**	**6.9**	**-1.42 ± 0.07**	**0.238 ± 0.009**
5.	AA-5	783	7.0	-1.62 ± 0.15	0.248 ± 0.012
6.	AA-6	794	7.3	-1.52 ± 0.14	0.249 ± 0.038
7.	AA-7	826	6.9	-1.58 ± 0.08	0.268 ± 0.025
8.	AA-8	832	7.0	-1.52 ± 0.09	0.278 ± 0.021
9.	AA-9	855	7.9	-1.47 ± 0.13	0.289 ± 0.032

***In-vitro* drug release study of *Achyranthes aspera* extract loaded nanogel:**

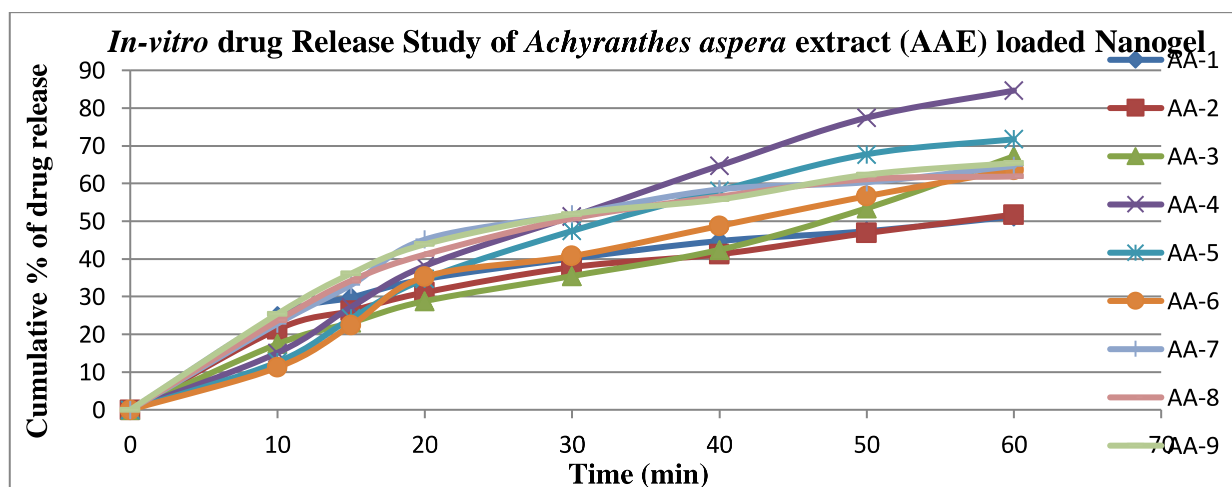

Figure 7: *In-vitro* drug Release Study of *Achyranthes aspera* extract (AAE) loaded Nanogel

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In-vitro drug Release of *Achyranthes aspera* extract (AAE) loaded Nanogels was revealed that the first three formulations (AA-1 to AA-3) showed increasing drug release percentage, formulations (AA-4 to AA-6) showed decreasing drug release percentage while formulations (AA-5 to AA-9) showed about similar drug release. It is because of concentration of hyluronic acid.

Drug release Kinetics:

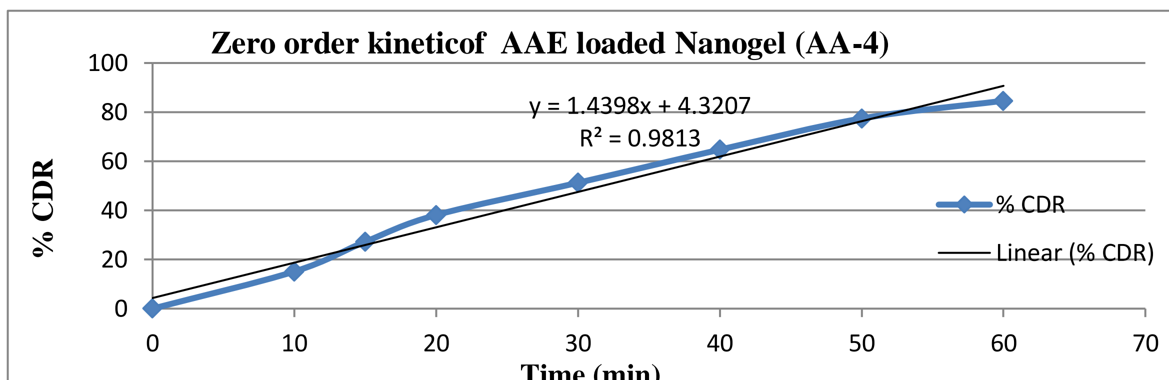

Figure 8: Zero order kineticof AAE loaded Nanogel (AA-4)

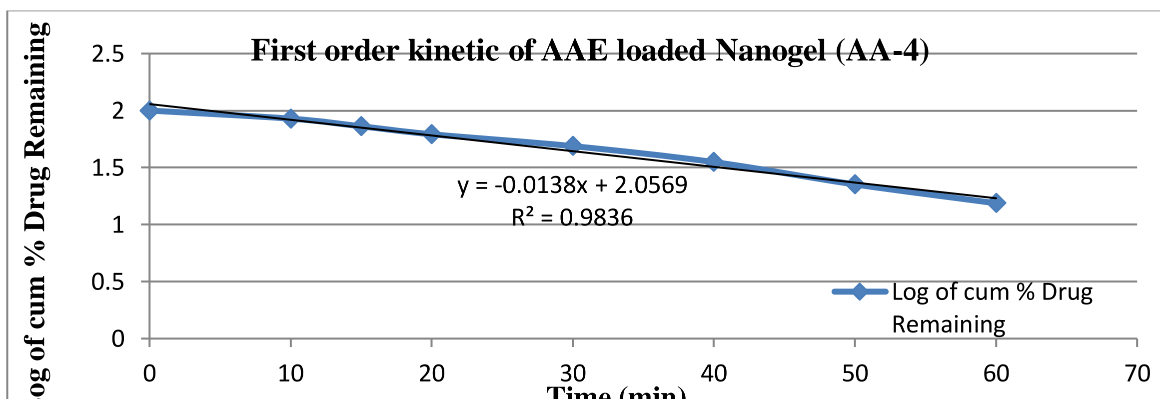

Figure 9: First order kinetic of AAE loaded Nanogel (AA-4)

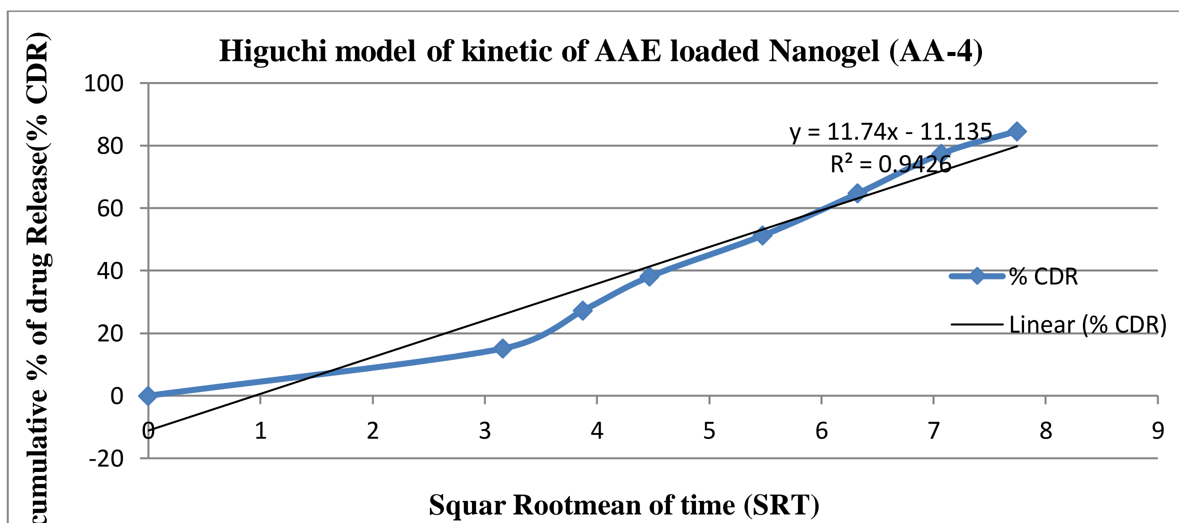

Figure 10: Higuchi model of kinetic of AAE loaded Nanogel (AA-4)

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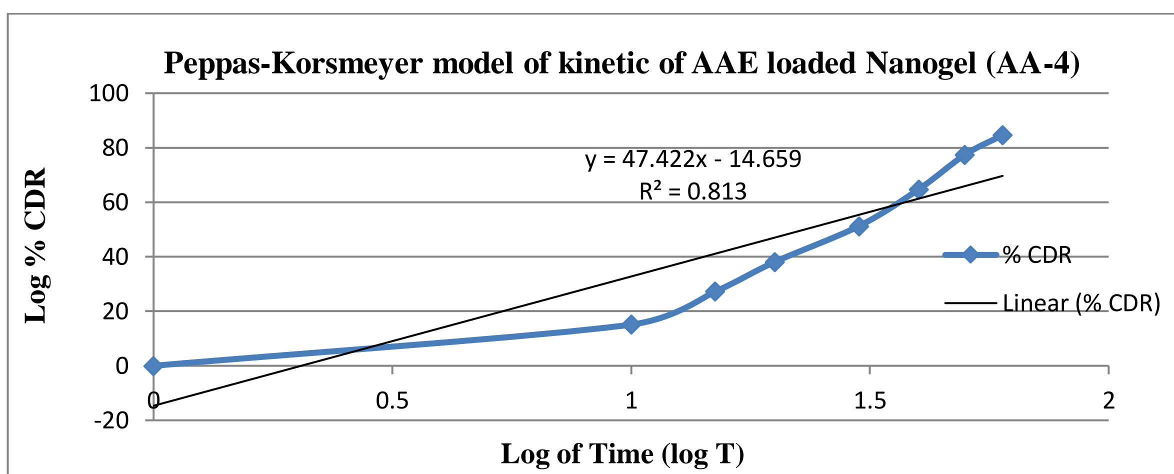

Figure 11: Peppas-Korsmeyer model of kinetic of AAE loaded Nanogel (AA-4)

Stability Study

Table No. 6: Stability of *Achyranthes aspera* extract loaded nanogel (AA-4)

Parameters	**25°C ± 2°C temperature/ 75% ± 5% R. H. (Refrigerator condition Accelerated study)**				
	Initial Values	**1 Month**	**2 Month**	**3 Month**	**6 Month**
Appearance	Clear Transparent	Clear Transparent	Clear Transparent	Clear Transparent	Clear Transparent
pH	6.9	6.9	6.9	7.1	7.1
Particle Size (nm)	302 ± 12	305 ± 21	307 ± 34	309 ± 52	315 ± 05
% Drug entrapment	89.92 ± 0.92	89.42 ± 0.42	90.62 ± 0.68	86.73 ± 0.13	84.16 ± 0.25
Viscosity	760	764	762	770	791

All values are mean of triplicate value (n=3) ± S.D

Antioxidant activity of *Achyranthes aspera* Extract (AAE)

DPPH scavenging activity:

Table No. 7: DPPH scavenging activity as the % inhibition

Sample	**% inhibition of DPPH at different Conc. (µg/ml)**						**IC_{50} Value (µg/ml)**
	0	**100**	**200**	**300**	**400**	**500**	
Ascorbic acid	**0**	53.64	63.42	78.41	88.52	99.81	< 100
***A. aspera* extract**	0	21.83	32.54	45.23	57.23	71.56	> 300
Nanogel AA-4	0	31.25	45.63	62.47	71.37	84.73	> 200

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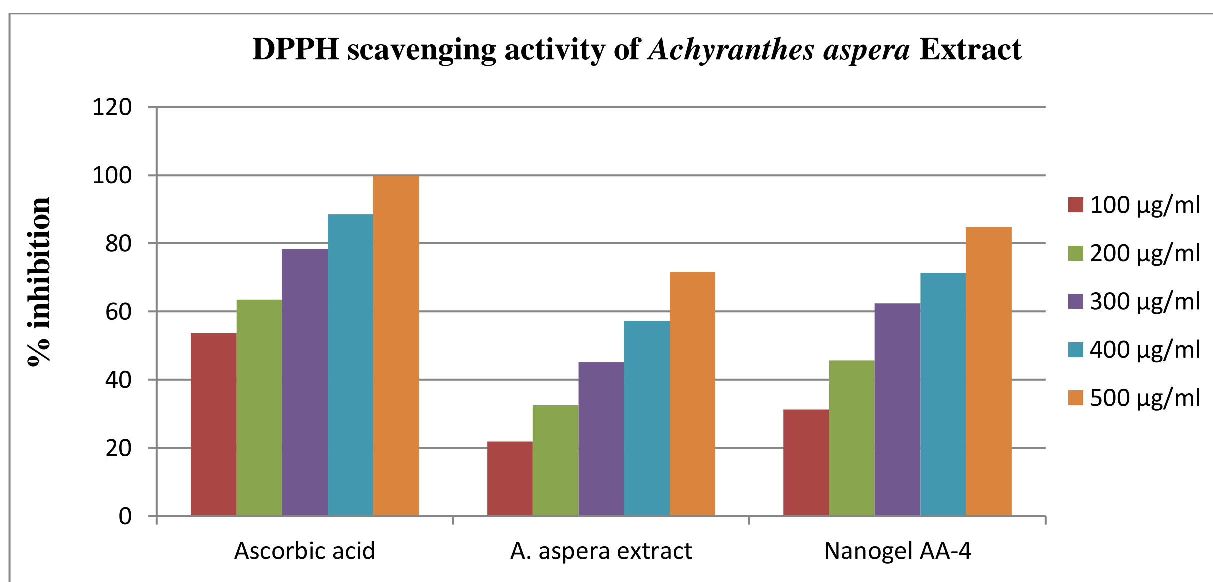

Figure 12: DPPH scavenging activity as the % inhibition

ABTS radical Reducing method:

Table No. 8: ABTS radical scavenging activity as the % inhibition

Sample	% inhibition of ABTS at different Conc. (µg/ml)						IC_{50} Value (µg/ml)
	0	100	200	300	400	500	
Ascorbic acid	**0**	52.33	62.74	76.53	89.68	97.89	< 100
***A. aspera* extract**	0	23.64	36.45	46.87	58.67	72.19	< 400
Nanogel AA-4	0	32.84	47.25	53.74	67.94	85.23	< 300

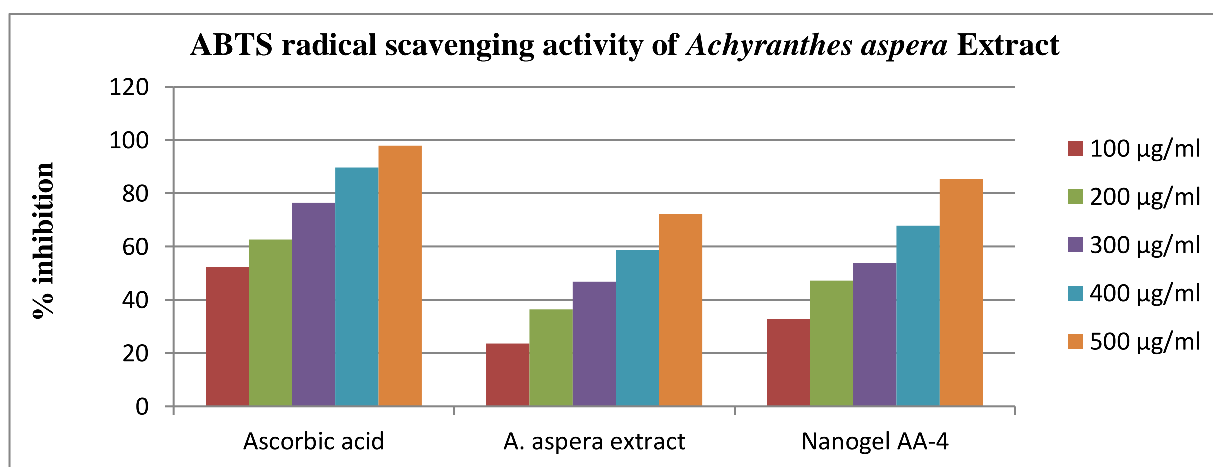

Figure 13: ABTS radical scavenging activity as the % inhibition

The findings for the DPPH and ABTS assay showed that the IC_{50} of the *Achyranthes aspera* Extract and the prepared nanogel (AA-4) have 2 to 4 fold poor antioxidant activity then the standard ascorbic acid (IC_{50} =100µg/ml). Also, concluded that it has poor anti aging activity.

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***In-vitro* antimicrobial evaluation of prepared optimized formulations**

The *A. aspera* extract and Nanogels (AA-4) have greater Antibacterial potential against *P. acnes.*

Figure 14: Antibacterial activity of *A. aspera* extract and Nanogels (AA-4) against *P. acnes*

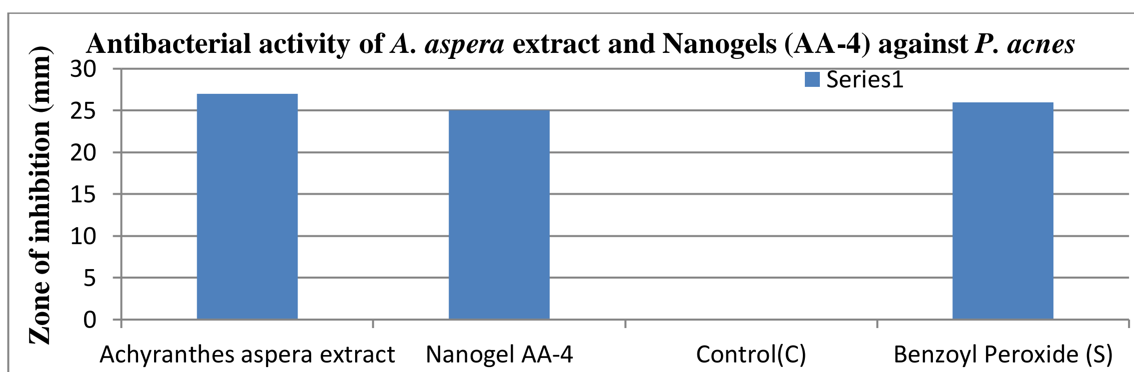

Figure 15: Antibacterial activity of *A. aspera* extract and Nanogels (AA-4) against *P. acnes*

Determination of *in-vivo* anti-inflammatory activities using Croton Oil induced ear edema model

Table No. 9: Determination of *in-vivo* anti-inflammatory activities by weight difference

Groups	Sample	Final (9 day) weight in mg		
		Left ear Control (mg)	Right ear (mg)	% difference in weight
Group-I	2.5% Croton Oil (placebo)	104 ± 2	168 ± 1	38.095 ± 2.2
Group-II	Dexamethasone (S)	101 ± 3	102 ± 2	00.980 ± 1.4
Group-III	*A. aspera* extract	102 ± 1	126 ± 1	19.047 ± 1.5
Group-IV	AA-4	112 ± 3	117 ± 2	04.274 ± 1.7

Table No. 10: Determination of *in-vivo* anti-inflammatory activities by thickness of ear edema

Groups	Sample	Final (9 day) thickness in mm		
		Left ear Control	Right ear	% difference in thickness
Group-I	2.5% Croton Oil (placebo)	1.5 ± 0.02	3.8 ± 0.02	60.526 ± 1.3
Group-II	Dexamethasone (S)	1.6 ± 0.05	1.7 ± 0.01	05.882 ± 2.1
Group-III	*A. aspera* extract	1.2 ± 0.04	1.6 ± 0.04	25.000 ± 1.7
Group-IV	AA-4	1.4 ± 0.01	1.5 ± 0.03	06.667 ± 1.0

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JCHR (2024) 14(5), 1973-1986 | ISSN:2251-6727

In-vivo anti-inflammatory activity of prepared optimized Nanogel formulations using Croton Oil induced ear edema model was performed on mice. *In-vivo* anti-inflammatory activities was determined by the % weight difference in controlled left ear and treated with nanogel right ears of respective groups. Results reveals that the 2.5% Croton Oil (placebo) treated Group-I showed 38.095 ± 2.2% weight difference to controlled left ear and Dexamethasone treated positive controlled Group-II was showed minor % difference in weight (00.980 ± 1.4 %). While, among prepared nanogels *A. aspera* extract loaded nanogel (AA-4) possessed greater potential against inflammation with % difference in weight (04.274 ± 1.7). Other prepared nanogels and extract showed moderate activity against inflammation. *In-vivo* anti-inflammatory activities was determined by the % difference in thickness in controlled left ear and treated with nanogel right ears of respective groups. Results reveals that the 2.5% Croton Oil (placebo) treated Group-I showed 60.526 ± 1.3% difference in thickness to controlled left ear and Dexamethasone treated positive controlled Group-II was showed minor % difference in thickness (05.882 ± 2.1%). While, among prepared nanogels *A. aspera* extract loaded nanogel (AA-4) possessed greater potential against inflammation with % difference in thickness (06.667 ± 1.0). Other prepared nanogels and extract showed moderate activity against inflammation.

Biochemical test for thiobarbituric acid reactive substances (TBARs) and thiol groups

Table No. 11: Effect of extracts and nanogels on TBARs and thiol groups levels in edema induced mice ears

Groups	Sample applied	TBARs (MDA equivalents/ear) µmol/L		Thiol groups (TNB equivalents/ear) µmol/L	
		Left ear Control	Right ear	Left ear Control	Right ear
Group-I	2.5% Croton Oil	0.53 ± 0.02	2.01 ± 0.03	12.53 ± 0.01	0.75 ± 0.01
Group-II	Dexamethasone	0.56 ± 0.05	0.68 ± 0.03	12.87 ± 0.04	12.43 ± 0.03
Group-III	*A. aspera* extract	0.51 ± 0.05	0.87 ± 0.01	12.66 ± 0.01	10.86 ± 0.02
Group-IV	AA-4	0.57 ± 0.03	0.72 ± 0.03	12.38 ± 0.01	11.64 ± 0.01

Histopathological Study

(A) Left ear (control group) section (B) Croton Oil treated inflamed section (C) Dexamethasone treated (D) *A. aspera* extract treated (E) Nanogel AA-4 treated. Presence of fibroblasts due hyperalgesia and the presence of infiltrated nuetrophils (small blue dot) indicated inflammatory cells.

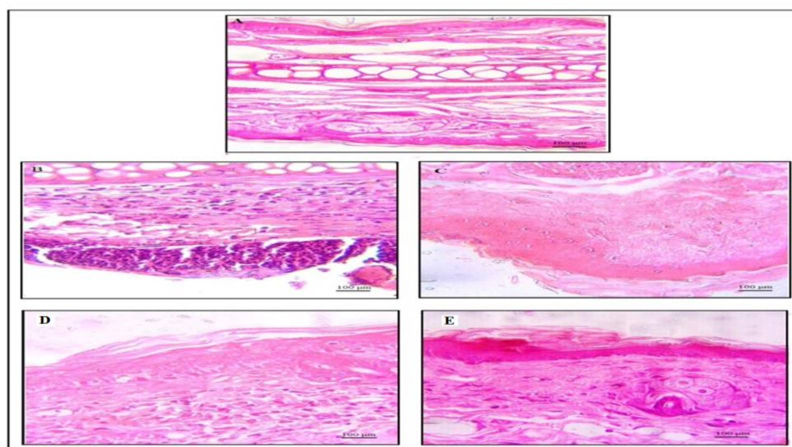

Figure 16: Photomicrographs (optical microscopy, 100X magnification, hematoxylin-eosin stain) of transverse sections of ear biopsies from mice after chronic inflammation induced by multiple applications of croton oil

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JCHR (2024) 14(5), 1973-1986 | ISSN:2251-6727

CONCLUSION

The investigation results of antioxidant activity of *Achyranthes aspera* extracts and extract loaded nanogels were performed as DPPH and ABTS radical scavenging activity. It was concluded that the *Achyranthes aspera* extracts and nanogel AA-4 possessed potential to scavenge free radicals it will helps to reduces aging because the main cause of aging is free radicals. It was also concluded that the developed nanogel provides unique mechanism to deliver drug effectively at inflamed ear of mice when applied topically. Both delivery and biological activity are significantly enhanced, which was found not to be pro-inflammatory. In a therapeutic context, this *Achyranthes aspera* loaded nanogel and *A. aspera* nanogel delivery system would potentially useful for the topical delivery of a drugs.

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