Sumerianz Journal of Medical and Healthcare, 2021, Vol. 4, No. 1, pp. 35-42
ISSN(e): 2663-421X, ISSN(p): 2706-8404
Website: https://www.sumerianz.com
DOI: https://doi.org/10.47752/sjmh.41.35.42

Original Article Open Access

A Novelistic Biomaterial from Natural Source: its Isolation and Physico-Chemical Characterization

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Article History
Received: February 3, 2021
Revised: March 7, 2021
Accepted: March 9, 2021
Published: March 11, 2021

Abstract

The objective of this research was to isolate the biomaterial from *Juglans regia* and explore its different inbuilt polymeric properties by performing different physico-chemical analysis. As we know there are a number of polymers available and frequently used in design and delivery of novel drug targeting tool or carrier systems. But isolated biomaterial from natural source have proved about their novelistic different properties like biodegradable, bioretardant, bioadhesive, filmability etc. These properties are the key factor in design the novel drug targeting tool. So these polymeric properties identified in the natural source biomaterials have drawn the attention of researchers. So in this study the isolated biomaterial was subjected to various physicochemical evaluations along with spectral analysis including UV, FT-IR, Mass and 1H NMR, SEM, rheological characteristics and cell-line toxicity study The isolated biomaterial was found tom be polymeric in nature having a numerous functional properties. On the basis of its inbuilt polymeric properties, the biomaterial isolated from *Juglan regia* seed, can be used as an alternative to available standard polymers at very economical scale. The isolated biopolymer consisted of a unique polymeric properties similar to available standard polymers.

Keywords: Biomaterial; Biopolymer; Bionanoparticles; Bionanosuspension; Juglans regia seeds.

1. Introduction

Polymers are the boon in field of drug delivery systems. These have been proved as the backbone for drug delivery systems designing. These play a very important role in designing of novel drug delivery systems to overcome a number of complications in drug delivery system designing [1]. These are used for controlling the release of the drug in desired manner. The hydrophilic and lipophilic polymers are the first choice for getting the desired release in controlled, sustained, extended, pulsed, prolonged release at the desired sites. Apart of this, these synthetic and semisynthetic polymers are produced by a number of chemical reactions and purification processes. Since these are produced by a number of unit processes which are costly.

Now days a number of researches are under investigation for avoiding the environmental, physiological and economical issues associated with the synthetic and semisynthetic polymers. So an alternative to synthetic and semisynthetic polymers are under investigation having novelty, potentiality, and all other benefits with minimum adverse effects on environment and physiology of the human beings.

One of the alternatives to synthetic and semisynthetic polymers is biopolymers [2] which have drawn the attention of researchers in designing of novel drug delivery design.

Biopolymers are novel, intelligent and smart polymers which have been isolated from many natural sources [3].

The nanoparticles are one of the most preferred and advanced carrier systems [4] for the treatment of CNS disorders like epilepsy, brain tumors etc. Since nanoparticles [5, 6] are can easily reach to brain after crossing the BBB and significant concentration of drug may be achieved for prolonged time. So the nanoparticles prepared by using novel isolated biopolymer are referred as bionanoparticles as these are biocompatible and biodegradable with efficient drug targeting [7-9].

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In this way the isolated biopolymer [10, 11] from the natural source like seeds of *Juglansregia*. So isolated biopolymer [12] from *Juglans regia* may be used as an alternative of available synthetic and semi synthetic polymers. This isolated biopolymer is biodegradable and biocompatible.

2. Materials and Methods

The ***Juglans regia*** (seeds/kernels) was purchased from the market of Lucknow. All other chemicals used were of analytical grade.

2.1. Isolation of Biopolymer

The ***Juglans regia***seeds were purchased from the local market of Lucknow. 200 gram of seed was weighed and soaked in double distilled water overnight. The swollen seeds were taken and their outer covering was removed. The uncovered seed was grinded in grinder as a paste. If necessary small quantity of distilled water (100ml) was added during the grinding. This paste as a slurry was filtered through the muslin cloth. The collected filtrate was centrifuge at 5000rpm for 10 minutes. After centrifugation the supernatant was taken. Centrifugation was done to remove any residue. Then half of the supernatant was treated with acetone in 1:1, 1:2 and 1:3 ratios. Another half of supernatant was treated with the methanol in the same ratios as acetone. Then these were place in refrigerator for overnight. Then after treatment these were centrifuged at 5000rpm for 30 minutes. The supernatant was discarded and the biomaterial as a sediment collected and air dried. If any moisture is there can be dried in desiccators for 48 hours. If the biomaterial consists of any oil, can be removed by washing with acetone or chloroform. This procedure for isolation was optimized by repeating six times and then percentage yield was calculated. The obtained biomaterial was passed through sieve number 200 and stored for further use [13-15].

2.2. Yield of Isolated Biomaterial

The isolated biopolymer was calculated for their %yield. This was calculated six times (n=6) with the help of digital balance (Labmann). This was calculated on the basis of weight of the isolated dried biopolymer powder obtained to the amount of the raw material used for extraction process ratio multiplied with 100. The total weight of isolated biopolymer was noted and %yield was calculated with standard deviation. The %yield was calculated by dividing weight of dried biopolymer powder with the weight of raw materials used and multiplied with 100.

2.3. Characterization of Isolated Biopolymer

The physico-chemical properties of isolated biopolymer were characterized [11] for color, odor, taste and solubility. The chemical tests for presence of carbohydrate and proteins were also performed. The isolated biopolymer was also characterized for SEM analysis, DSC testing, FTIR spectroscopy, mass spectroscopy and NMR spectroscopy [15].

2.4. Physico-Chemical Characterization of Biomaterial

The color, odor, texture of isolated biopolymer were physically evaluated. The color changing point was determined by using the melting point test apparatus. The isolated biopolymer was filled in the capillary tube and it was kept in melting point apparatus. The apparatus was switch on and observed for the temperature at which there was change in the color and melting of biopolymer starts. The temperature was observed with the help of thermometer [15]. The organoleptic properties like Color, odor and taste were observed. The pH was determined for 1% aqueous solution with the help of digital pH meter (Cystronics). The tests were performed in triplicate (n=3).

2.5. Solubility study of Biomaterial

The solubility study of the isolated biopolymers were performed in different solvents like distilled water, methanol, diethyl ether and acetone, the excess of the isolated biopolymer was added in 10ml of the specific solvent system in beaker gradually. The solution was dispersed well and kept for 24 hours on orbital shaker for achieving equilibrium state. Then the solution was centrifuged at 400rpm in centrifuge for 10 minutes and then filtered to get the clear solution. Then the filtrate was allowed for measurement in UV spectrophotometer machine (Mapada). The procedure was performed in triplicate for each isolated biopolymer [16].

2.6. Particle Size Analysis of Biomaterial

This was performed by using optical microscopy method. The isolated biopolymer was taken on the glass slide and added 1 drop of glycerin. The cove slip was placed on the drop and examined with the help of calibrated eye piece micrometer under the optical microscope. During examination about 100 particles were counted and particle size distribution was determined. This was performed in triplicate and calculated with the help of following formula:

$Xg = 10x [(ni \times \log Xi)/N]$

Xg is geometrical mean diameter,ni number of particles in range, Xi is the midpoint of range and N is a total number of particles

2.7. Determination of Rheological Properties of Biomaterial

2.7.1. Bulk Density

The bulk density of the isolated biopolymer was calculated by taking accurately pre-weighed biopolymer in measuring cylinder and then the bulk volume was measured. This was performed in triplicate [17].

2.7.2. Tapped Density

The tapped density of the isolated biopolymer was determined by taking a pre-weighed biopolymer in measuring cylinder and then tapped for 100 tapping. Then the tapped volume was determined. This was performed in triplicate. Then the tapped density was calculated [17].

2.7.3. Angle of Repose

Funnel method was used for determination of angle of repose. Accurately weighed powder material was taken in funnel. The height of funnel was adjusted so that it touches the tip of funnel just touches the apex of heap of blends. The blends were allowed to flow through funnel freely on the surface. This was performed in triplicate.The angle of repose was determined from the ratio of $\tan^{-1}$ of ratio of height of the pile and radius of pile. The obtained results were correlated [17].

2.7.4. Percentage Consolidation Index

It is used for determination of flow properties. It is very simple, fast and widely used method for determining powder flow characteristics.This was performed in triplicate. This was calculated as the ratio of subtracted value of tapped and bulk density with tapped density. According to Carr'sindex powder with 10% flowability is considered as excellent flow characteristics. Powder with less than 15 % flowability is considered as powder with good flow characteristics and above reveals about poor flow property [17].

2.8. Chemical Tests for Chemical Constituents

2.8.1. Chemical Test for Carbohydrate

1ml of biopolymer solution (5% biopolymer solution in distilled water) was taken in test tube. Add two drops of Molisch reagent .Add 1-2 ml of conc. Sulfuric acid in the test tube and observe for the formation of purple color at the at the interface of two layers formed [17].

2.8.2. Chemical Test for Proteins

Biuret test was performed for the confirmation of proteins. 2 ml of *Juglans regia* solution was taken in test tube (5% biopolymer solution in distilled water),add 1 ml of sodium hydroxide solution with addition of copper sulphate solution drops. The mixture was kept aside for five minutes and observe any color changes. The appearance of violet color confirms the presence of proteins [17].

2.8.3. Test for Reducing Sugar

The 2ml of biopolymer solution (5% biopolymer solution in distilled water) was taken in test tube followed by addition of 1ml each of Fehling's solution A and Fehling's solution B. The mixture was heated in water bath for few minutes in water bath and observes for precipitate. The brick red precipitate appearance after heating confirms the presence of reducing sugar in the isolated biopolymer.

2.8.4. SEM

The isolated biopolymer was analyzed by scanning electron microscope. In SEM analysis the external surface and internal structure was characterized. The small quantity of biopolymer was taken and fixed on aluminum studs and the coated with gold with the help of coater sputter under vacuum. Then the SEM image was observed for the sample under test [16, 17].

2.9. Spectral Analysis

2.9.1. FTIR Spectroscopy

The FTIR spectroscopy was done by preparing the KBr discs. 1mg of isolated biopolymer was taken and mixed with 100mg of dried and desiccated solid KBr (Potassium bromide). The mixture was mixed in mortar and pestle and placed in IR lamp to remove any moisture. The mixture was converted into disc by using the hydraulic pump under the pressure of 10 tons. The prepared KBr disc was placed in disc holder in the path of IR radiation [16]. The spectrum was recorded into the range of 4000-200cm^{-1}.

2.9.2. Differential Scanning Calorimetry

In DSC testing is the thermal analysis technique in which the heat flow into or out of the sample is determined as the function of temperature. Here the sample was taken and exposed to controlled temperature program. The glass transition temperature was determined. The heat flow range was 50-300℃. The DSC thermogram was recorded [11, 16].

2.9.3. Mass Spectroscopy

This is the laboratory technique in which the sample of biopolymers was introduced in the through the inlet system. The gas phase ions of the compound were produced. Then molecular ion fragmentation, the ions separated in mass spectrometer according to their mass to charge ratio. A mass spectrum of ion abundance versus mass to charge ratio was obtained [11, 16].

2.9.4. NMR Spectroscopy

The NMR spectroscopy was done for spectral analysis of isolated biopolymer. The sample was dissolved in specific solvent like CDCl3. The mixture was pumped in the instrument at high rate flow. The valve switch was used to stop the flow. The measurement was performed. After the finishing of measurement the spectrum was processed and analyzed in automation computer [11, 16].

2.10. Cytotoxicity Evaluation of Bio-Polymer on Neuroblastoma Cell Line

Cytotoxicity evaluation of isolated bio-polymer was done on Neuroblastoma cell line. The materials used are Cell line –SHSY-5Y,(human breast cancer cell line), Ham F-12 media, fetal bovine serum(FBS),antibiotic-antimicotic solution from thermoscintific and MTT reagent from sigma Aldrich, USA. Tissue culture flask, six well micro-culture plates from Eppendorf, Germany. In methods maintenance of cell lines, subculturing process of cell lines, trypsinization, cryopreservation of cell lines was done. In MTT assay the formazan product is analyzed spectrophotometrically (540nm) after dissolution in DMSO, the spectra of treated and untreated cells giving an estimate of the extent of Cytotoxicity [15].

3. Results and Discussion

3.1. Appearance, %Yield, Color Changing Point of Isolated Biomaterial

The isolated ***Juglans regia*** biopolymer showed the whitish-cream in color. The %yield of isolated biopolymer was found to be 8±1.2% .The color changing point was found to be 275℃±4℃. This means that a significant yield was isolated from the natural seeds [11, 16].

3.2. Characterization of Isolated Biopolymer

As the isolated biopolymer (appears as whitish-creamcolor in appearance. In physico-chemical characterization the biopolymer was found to be odorless with characteristic taste. The isolated biopolymer was soluble in water and methanol and found to be Insoluble in acetone and diethyl ether [15, 16].The tests for Carbohydrate and protein showed positive test in chemical testing for these constituents. The presence of these high molecular weight constituents reveals that these are polymeric in nature. The observation of different Characterized parameters findings of isolated biopolymer of***Juglans regia***is shown in Table 1.

Table-1. Characterization of Isolated Biopolymer of Juglans regia

Parameters evaluated	**Observation**
Color	White-cream
Odor	Characteristic
Taste	Characteristic
Melting Point	275℃±4 ℃.
Solubility	Soluble in water and methanol
	Insoluble in acetone and diethyl ether
Carbohydrate	Present
Protein	Present

3.3. Particle Size Analysis of Biomaterial

The particle size of the isolated biopolymer was to be found in the range of 54.32-314.8µm. The result reveals that the biopolymer is of granular in nature with flaky appearance with different particle size. The flaky appearance with granular structure was also correlated with SEM image. The particles observed were found to be similar to the standard polymers [16].

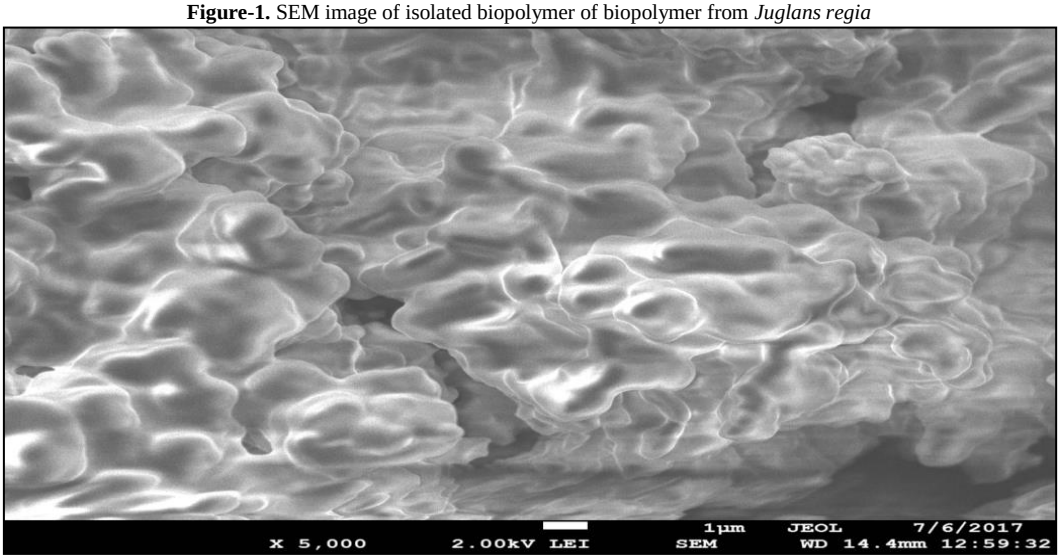
3.4. Rheological Properties of Biomaterial

The different rheological characterization like bulk density was found to be 0.66±012 g/cm3, tapped density 0.9±0.11g/cm3, cars index-13.5±1.2% which showed the satisfactory results. Thus the isolated biopolymers were found to be free flowing and are suitable for the preparation of bionanosuspension [11, 16].

3.5. SEM

The SEM (scanning electron microscopy) analysis of the isolated biopolymer form *Juglans regia* showed the flaky and granular surface. Such granular and flaky structures confirm its polymeric nature [11]. The SEM image of isolated biopolymer has been shown in Figure 1.

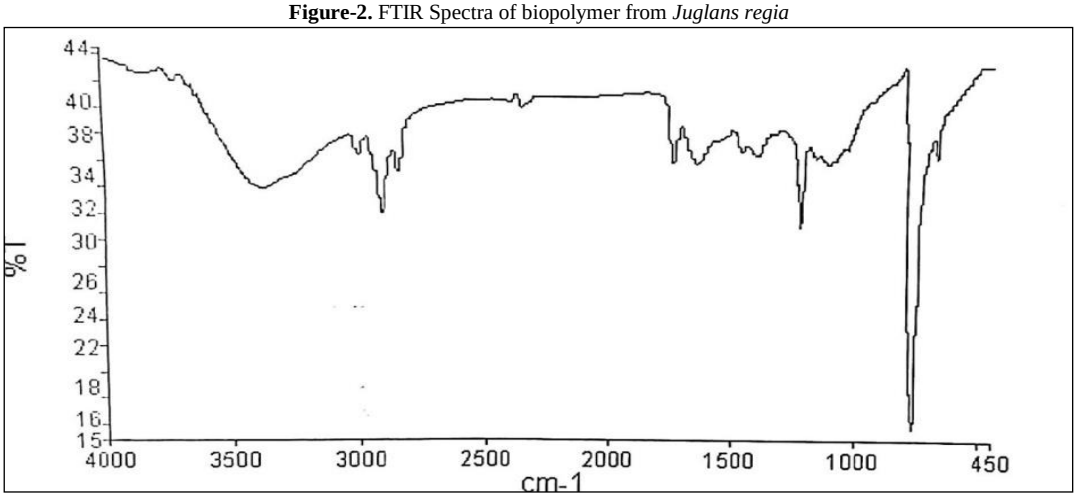
Figure-1. SEM image of isolated biopolymer of biopolymer from *Juglans regia*



3.6. FTI.R. Spectral Characterization

The I.R. Spectral analysis of biopolymer reported the presence of functional groups like hydroxyl (3395.29cm^{-1}), alkynes (668.01cm^{-1}),carboxylic acid (1386.63cm^{-1}) and also other groups like amide at 1638.82 cm^{-1} , alkenes at 2926 cm^{-1} [11]. The Presence of these functional groups is responsible for its polymeric nature like other synthetic and semisynthetic polymers [11, 16]. FTIR spectra has been shown in Figure 2.

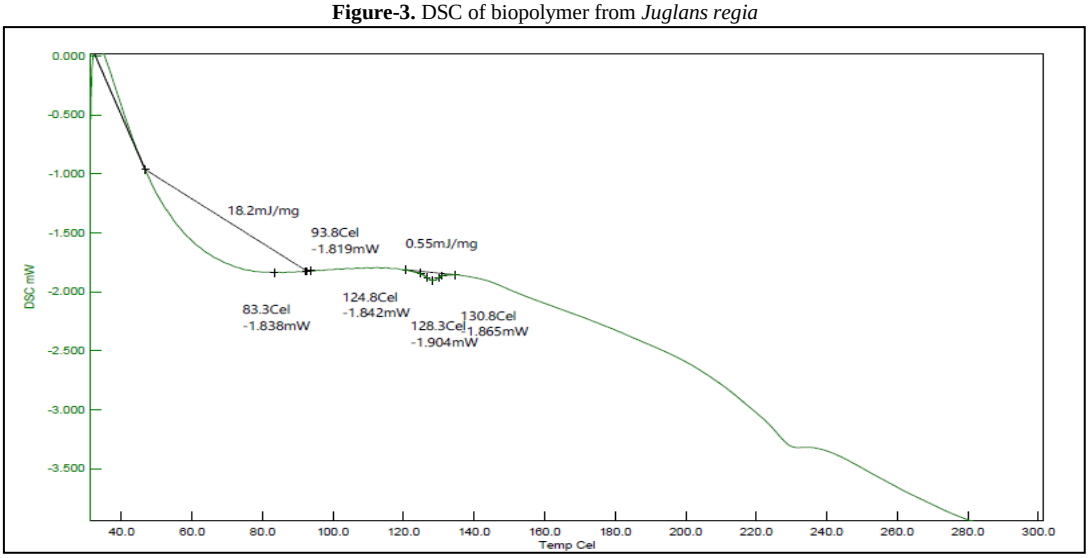
Figure-2. FTIR Spectra of biopolymer from *Juglans regia*



3.7. Differential Scanning Calorimetry

The DSC of ***Juglans regia***shows peaks at 83.27Celand 128.3Cel. The area was found to be 18.24 mj/mg and 0.55 mj/mg respectively [11]. The obtained result confirms its polymeric nature. The broad endothermic peak reveals its polymeric nature as shown in Figure 3.

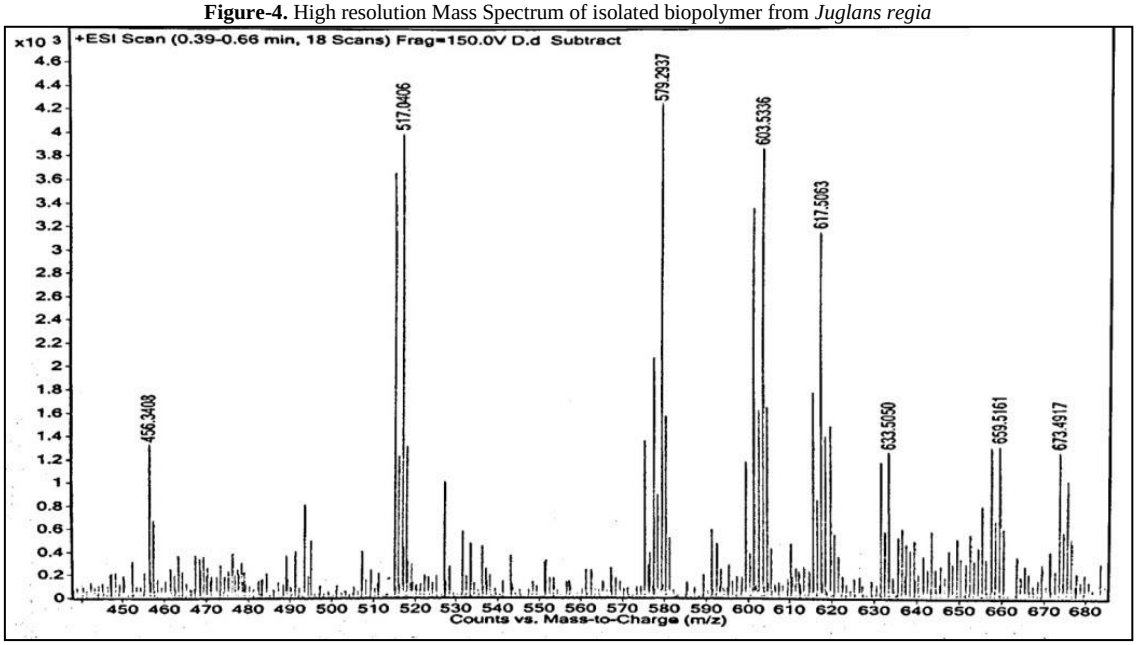
Figure-3. DSC of biopolymer from *Juglans regia*



3.8. Mass Spectroscopy

Mass spectral analysis of the isolated biomaterial reveals that the isolated biopolymer is polymeric in nature due to presence of high molecular weight structure. The presence of the high molecular weight confirms the presence of proteins. The high resolution mass spectra of isolated biopolymer showed the parent peak at m/j 579.29 [11]. Its large molecular weight structure like proteins which indicates its polymeric nature as shown in Figure 4.

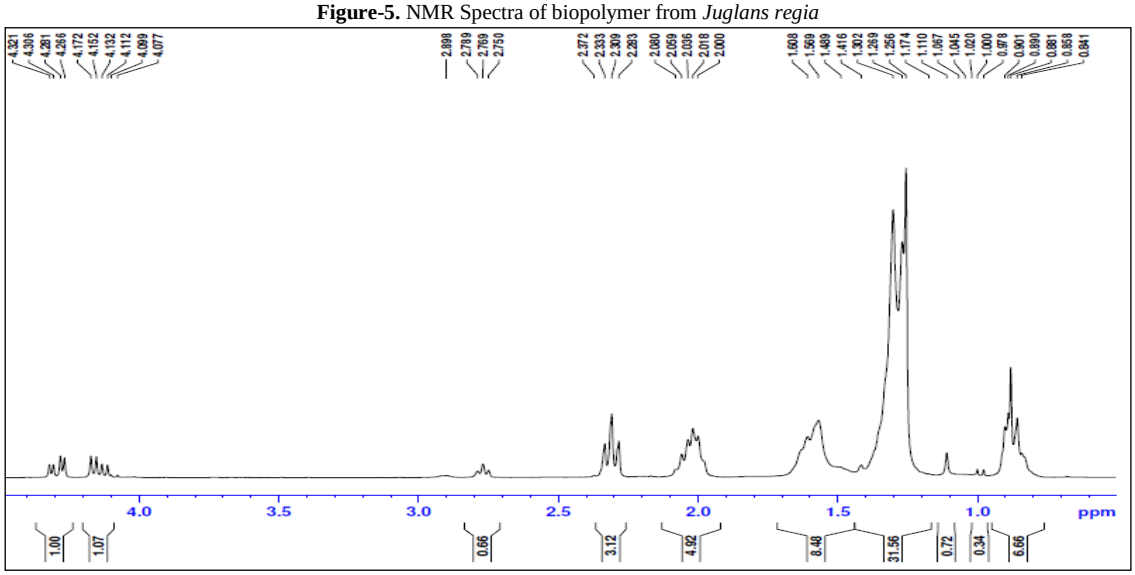
Figure-4. High resolution Mass Spectrum of isolated biopolymer from *Juglans regia*



3.9. NMR Spectroscopy

The NMR spectra shows the presence of peaks like peak at 0.90ppm which reveals the presence of primary alkyl group, peak at 1.25ppmconfirms the presence of methylene group, at1.26ppm shows the presence of hydroxyl group, at 2.3ppm confirms about the presence of ester group, at 4.2 reveals about aliphatic methylene proton [11, 16]. The presence of these groups confirms its polymeric nature as given in Figure 5.

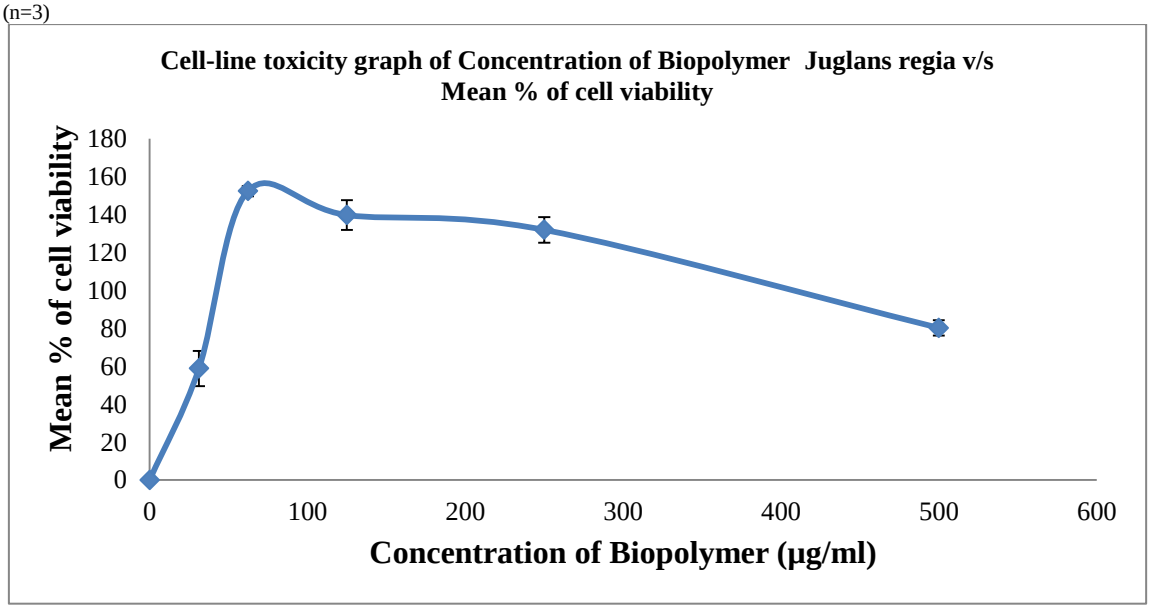
Figure-5. NMR Spectra of biopolymer from *Juglans regia*



3.10. Cell line Toxicity Study of Juglans Regia

The cell line toxicity study of Juglans regia biopolymer in the concentration of 0.31.25, 62.25, 125,250 and 500 (µg/ml) shows the mean % cell viability ranging from 152.38±2.72% to 58.843±9.27%with IC50 values (µg/ml) of >500µg/ml. Thus the cell viability assay data demonstrate that there is no cell death observed in assay. Along with this the IC50 value of the biopolymer was above 100µg/ml. So the obtained data revealed that biopolymer was found to be safe and non-toxic in nature. So it can be safely used for the preparation of drug loaded bionanosuspension [15]. Cell-line toxicity graph of Concentration of Biopolymer *Juglans regia* v/s Mean % of cell viability has been shown in Figure 6.

Figure-6. Cell-line toxicity graph of Concentration of Biopolymer *Juglans regia*v/s Mean % of cell viability. The results are expressed as mean ± SEM (n=3)



4. Conclusion

In this research the bionanosuspension loaded with phenytoin were developed by using the biopolymer isolated from the seeds of *Juglans regia*. The obtained outcomes reveal that the isolated biomaterial having a number of such promising characteristics may be used as a novel biomaterial for designing of bio-nanoformulations. Biopolymer from natural sources stands as an alternative to standard synthetic and semisynthetic polymers because of its biodegradability, biocompatibility, [15] with a number of inbuilt properties. Since biopolymer one of the excellent biomaterial which is present in natural resources but its novelty has been explored in broad. So the biopolymer can be safely used as the novel biomaterial for developing bionanosuspension in delivery of nanosized phenytoin to the target site for long term treatment of epilepsy [11, 15, 16].

The biomaterial was isolated from Juglans regia and has been tested to various physicochemical appraisals close by ridiculous assessment including UV, FT-IR, Mass and 1H NMR. The biomaterial from Juglans regia was found to be polymeric in nature, having different utilitarian properties. In light of its inbuilt polymeric properties, the biomaterial separated from different sources, can be used as an alternative as opposed to open standard polymers at incredibly proficient efficient scale. The isolated biopolymer contained an intriguing polymeric properties like available standard polymers. The confined biomaterial from normal sources shown particular inbuilt polymeric properties by execution of different physico-substance assessment. As we presumably know there are different polymers open and customarily used plan of novel drug. However, isolated biomaterial from Juglans regia exhibited its novelistic polymeric properties. In this way it very well may be successfully utilized as an option in contrast to accessible standard polymers in design of novel drug delivery systems.

Acknowledgements

I wish to acknowledge Prof. Devender Pathak (Dean, Faculty of Pharmacy, UPUMS, Saifai), for encouraging me for the completion of research work. I want to also thank to SAIF, CDRI, Lucknow for providing me analytical testing facilities.

Conflict of Interest

There was no any conflict of interest with this publication. There was no any financial support.

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