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IN VIVO ANTI-PYRETIC AND ANTI-INFLAMMATORY ACTIVITY OF CHITOSAN-REDUCED SILVER NANOPARTICLES USING MICE MODELS

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ABSTRACT

From this research the synthesized chitosan reduced AgNPs were examined by spectroscopic methods, AgNPs are primarily spherical with some degree of agglomeration, according to SEM examination. The effective synthesis is confirmed by the homogeneous distribution of nanoparticles with diameters in the nanometer ranges was observed at 70-100 nm, the produced nanoparticles showed a moderately polydispersed system with a Z-average size of 394.1 nm. With an intensity-based mean size of 273.3 nm, the effective synthesis of AgNPs is evident from the presence of a significant silver (Ag) signal with a weight percentage of 46.59% and an atomic percentage of 12.51%.Furthermore, components that correlate to the chitosan capping and stabilizing agent utilized in the synthesis are seen, including carbon (C), nitrogen (N), and oxygen (O). Remaining ions from the reaction medium may be the cause of the potassium (K) presence. XRD analysis confirmed the amorphous nature of the chitosan matrix is shown by the wide diffraction peak about 23.99° (2θ) in the XRD study of chitosan-mediated AgNPs. The lack of high and sharp peaks indicates that the silver nanoparticles are stable and well-embedded in the chitosan polymer, potentially in a semi-crystalline or amorphous phase as a result of chitosan capping, nano-drugs was checked invio in mice model against anti-inflammatory and anti-pyretic Activity they showed active potential over a 3-hour period they are investigated which works in one hour against inflammation 33.35 % and 30.19 % against pyrexia.
Key Word: *Silver-nanoparticles, Chitosan, Anti-inflammatory, Anti-pyretic, Reducing agent*

INTRODUCTION

In the scientific and technological community, Sustainable nanotechnology is effective at giving information to problems in a number of fields which, including agricultural, industrial, medical, and catalysis. (Beyene *et al.*, 2017). Owing to their tremendous qualities, It is utilized in many different items and sectors, including food, pharmaceuticals, consumer goods, optical sensors, medical device coatings, and cosmetics. Additionally, it has been used as a possible antibacterial agent in home, industrial, and medical items. (Zhang *et al.*, 2016).

Positive outcomes have been achieved when incorporating silver nanoparticles (AgNPs) into cancer treatments for targeted tumor therapy (Wei *et al.*, 2015). Commercially, AgNPs are employed as wound healers, immune adjuvants, biosensors, and anti-inflammatory medicines. AgNPs' excellent stability, biocompatibility, and capacity to incorporate both hydrophilic and hydrophobic molecules are among their primary technical

benefits for usage as drug carriers. Numerous methods, such as oral and inhalation, can be used to give the medications. (Karthik *et al.*, 2018).
Currently, medicinal polysaccharides, the chitosan have been analyzed to act as promising bio-reducing agent and act as a medicine in many others diseases, which can be utilized to formulate effective AgNPs. Previously, chitosan has been used in medicinal formulation to fabricate anti-microbial films in food packaging plus coating in drug delivery system. Chitosan being a natural polymer have proven to have excellent therapeutic capacity which use as anti-tumor in many patient, also use against inflammation and microbes, having no side effects (Mumtaz *et al.*, 2023).
According to Center of Disease Control and Prevention, a big portion of earth living people is afflicted by minor converted by major diseases. The most common affliction that irritates the body is inflammation and pyrexia. There are numerous allopathic medications that target either the infection or the symptom of pyrexia.
The difficulty in controlling and treating pathogenic vectors has increased due to drug resistance. However, due to limited access to synthetic pharmaceuticals, many marginalized individuals are forced to cure numerous diseases with locally available natural resources such as plants, fungi and beneficial bacteria. Advances in phytochemical research and nanotechnology are also Capturing the interest of researchers looking to identify new anti-pyretic and anti-inflammatory agents (Chaudhary *et al.*, 2023). The researchers have been currently investigating the utilization of AgNPs in medicine to treat and manage a wide range of ailments. With AgNPs, accounting for 55.4% of all nonmetals, it is also among the noble metallic NPs that have attracted the greatest interest and demonstrated the highest level of commercialization (Naganthan *et al.*, 2022).
Hence, in view of novel therapeutic properties of AgNPs the researcher study is designed to synthesize eco-friendly Silver nanoparticles act chitosan as bio-reducing and bio-capping agents. Furthermore, anti-inflammatory and anti-pyretic activity of AgNPs will be assessed using mice models. Nano technology is used for the betterment of humanity this concept of Nano technology arise from the Nano size10-100 type of material which is mainly used in medicine and engineering and also use in agriculture. (Tayo *et al*, 2017).
The Nano-devices are now a days mostly used cosmetology and chemotherapy, they are mainly called as umbrella form because they are unifies biological medicine that concepts of chemistry .mainly in physics and also in engineering site. (Xiao *et al.*, 2019). It has also be used now in many diagnostic purposes and conducting inks, image devices, fertilizer and pesticides also used as biosensor (Khan *et al.*, 2019).
The previous methods for formulated the nanoparticles are physical and chemical, mainly used meals for nanoparticles are gold, platinum, titanium and copper ,but the silver are specially examine to act and showing unique properties against microbes and antipyretic (Ramalingam *et al.*, 2014). After the used of Au ion is determine that the gold ion is excellent work as antimicrobial and antipyretic properties and also work as antioxidant also work against anti-viral and anti-cancer cells. (Gurav *et al.*, 2019).
To expedite a treatment of rheumatoid arthritis, the Au salts are also used against inflammation medications such as edema, When used within WHO-permissible levels, colloidal Ag can help to control the bone diseases, wrinkles of face, and the deformation of bone in old life (Kean *et al*, 2008).Additionally, Ag alloys to utilized as laminations for laser equipment, mostly in surgical site scissors, antimicrobial stents, pacemakers, and rarely in bandages to cure and control various microbial infections. (Lin *et al.*, 2009). When a chemical reducing agent was present, in the metal Ag salts work successfully reduced to synthesize the Ag nanoparticles. These reducing agents, which included sodium citrate and ethylene, sodium borohydride, diamante tetra-acetic acid, is extremely harmful for both human life health and the sustainability of the environment. Chitosan mediated AgNPs synthesis of Silver NPs as therefore faced to address the issue by bioreducing Silver ions ($Ag+$) to Silver nanoparticles (Ag) (Pissuwan *et al.*, 2019).
The current study was designed to use aqueous leaf extracts of A. modesta to physiologically decrease,

generate, and stabilize AgNPs in light of the plant's therapeutic qualities. The antibacterial, anti-leishmanial, and cytotoxic properties of Silver nanoparticles and plant aqueous leaf extract were then compared (Lin *et al.,* 2022). According to Klebowski et al. (2018),

Photosynthesized AgNPs have provided a new avenue for medication administration in the medical field. AgNPs' monolayer can help deliver the medication to specific locations, like proteins and nucleic acids. Additionally, the AgNPs can be physically adsorbed to the surface via ionic bonds or conjugated with the chosen medication using polyethylene glycol. Payload transport is examined in the presence of both in vitro (temperature) and in vivo (pH, cellular enzymes, and glutathione) stimuli (Hu et al., 2020). The plant, which is a member of the Mimosaceae family, is known locally as Palosa and Phulai. The entire plant—flowers, fruits, back, stem, roots, and leaves—contains therapeutic compounds that give it specific therapeutic properties. The plant is common throughout all of Pakistan's provinces, including KPK. (Abid *et al*., 2020).

Chitosan synthesized AgNPs is now day introduced method, Chitosan play a natural reducing agent to convert Ag^+ to Ag. They are easily available and quick work to prepared silver nanoparticles they are affordable and also use as waste product to minimize the environmental population (Ramalingam *et al.,* 2019).

AIM AND OBJECTIVES

The research study aims to synthesize cost-effective and therapeutically active AgNPs using chitosan as natural bio-reducing agent. Further, chitosan and synthesized AgNPs will be evaluated for in-vivo anti-inflammatory and anti-pyretic activity.

The objectives of the research are;

1. To characterize the chitosan reduced AgNPs using various spectroscopic instruments such as SEM, EDX, Zeta Sizer, XRD and FT-IR.
2. To analyze in-vivo the anti-pyretic and anti-inflammatory activities of chitosanreduced Silver Nanoparticles using mice models.

MATERIALS AND METHODS

Synthesis of AgNPs

First 30 minutes was be spent stirring 0.2 gram of chitosan in 1% acetic acid until it mix completely. Then it was be filtered to obtain a clear solution. In a sterile glass flask, 10 mL of the chitosan stock was react with 90 mL of AgNO3 (1 mm). After that mixture was be mixed with 100 µL of NaOH solution. Then the flask holding the reactant mixture was be keep in a water bath at 75 °C for 3 h the color Pale yellow to dark grayish black changes indicate strong AgNP production (Kalaivani *et al*., 2018).

Purification of Chitosan mediated AgNPs

After that process the Silver Nanoparticles was be dried using the spread-drying technique. When Silver Nanoparticles were dry then be removed with a scraper and submerged in purified water. After that, Centrifugation were be used to pellet the solution for 15 minutes at 12,000 rpm then discarding the pellets of purified AgNPs and the supernatants was be dried and keep at < 50 °C (Ahmad *et al.*, 2017).

Characterization of Chitosan mediated AgNPs

Zeta-Sizer

Zeta Sizer are important technique for AgNPs from which we was investigate using zeta-sizer to determine the size, zeta potential of the AgNPs The zeta potential and particle size of AgNPs at 25 °C and pH ˜7.0 were assessed using a Zetasizer Nano ZS (Malvern Panalytical, UK). Zetasizer software (v7.x) was utilized to assess the findings after polystyrene latex was employed for calibration.(Husseiny *et al.,* 2015).

Scanning Electron Microscopy of Chitosan mediated AgNPs

Using scanning electron microscopy techniques at various magnifications, the stability, shape, and surface area of AgNPs were examined using SEM (JEOL JSM-5910, Japan) at 30 kV with about 3 nm resolution.(Khatak *et al.,* 2019).

Dispersive Energy-Based X-Ray Spectroscopy

By the use of EDX, we can check AgNPs for the presence of functional components which we want with the use of EDX (INCA 200, Oxford Instruments, and UK) combined with SEM, AgNPs were verified. Silver and copper standards were used for calibration, and INCA software was used to evaluate the results. (Khatak *et al.,* 2019).

X-Ray Diffraction Measurements

A common technique which is use to find the average AgNPs particle size and the crystal lattice shape and Disparity that's all are examined with X-Ray Diffraction Measurements spectra was examined using XRD (JEOL JDX-3532, Japan) operating at 40 kV and 30 mA with Cu Kα radiation (λ = 1.5406 Å). MDI Jade software was used to evaluate the data after scans were conducted throughout the 2θ range of 10° to 80°. (Khattak *et al.*, 2019).

Fourier Transform Infrared Spectroscopy Chitosan mediated AgNPs

FTIR Spectroscopy is an important test use for characterization of AgNPs which find out the important functional group them findings qualitative and also quantitative functional groups to find functional groups, FTIR analysis was performed in the 4000–500 cm^{-1} range using a Bruker Alpha II spectrometer (Germany). The KBr pellet technique was used to prepare the samples, and OPUS software was used to analyze the results. (Khattak *et al.*, 2019).

INVIVO PHARMACOLOGICAL ACTIVITIES

Anti-inflammatory Activity of AgNPs (Carrageenan-induced paw edema)

After the completion process of AgNPs we can check in animal model, Initially, sub-acute inflammation was be induced/inject carrageenan (1%, 0.05ml), after that applied to the right hind foot to cause infection mice that had been starved overnight by sub-plantar injection on the first and third observation days. Before carrageenan induction, each mice's right hind paw was be marked at the level of the lateral malleolus so that, during the observation days, the mice's was always be submerged to the same degree in the plethysmometer measuring chamber. Plethysmometer are recorder of the daily volume of mice paw after one-hour extract, according to their respective groupings, the vehicle and the standard medication were administered. Drug and vehicle administration was started one hour before carrageenan injection and continued for seven consecutive days. After one week, the percentage of edema inhibition was be computed using the provided formula, for this activity male BALB-C mice around 25g weighted 3 groups of animal models provided in each group 7 mice were physically observed (Liao *et al*., 2012).

Anti-pyretic Activity of AgNPs

For the examine of our drug as anti-pyretic activity in mice model first Fever which cause by yeast (infectious fever) are cause in mice model this is the most usual and reliable technique AgNPs antipyretic potential was be examined using a model that was used to examine the antipyretic potentials of both natural and synthesized compounds male BALB-C mice around 25g weighted 3 groups of animal models provided in each group 7 mice the baseline rectal temperature were used to determined mice temperature every hours (Safaria *et al*., 2016).

RESULTS

Synthesis and Purification of AgNPs

Chitosan reduced AgNPs were effectively synthesized by having initial color change from pale yellow color to strong grayish black color and purified with the help of dry incubator by normal room temperature after the drying of AgNPs scrubbing and wash with purified water then centrifuge at 12,000 rpm for 15 minutes, dry and store in small pellets which is clearly shown in Figure 4.1, Figure 4.2 and Figure 4.3.

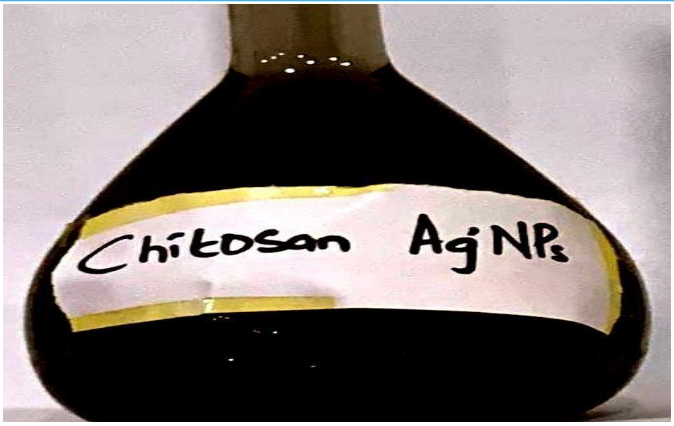


Fig. 4.1. Chitosan Mediated AgNPs


Fig. 4.2. Purification of Chitosan Mediated AgNPs

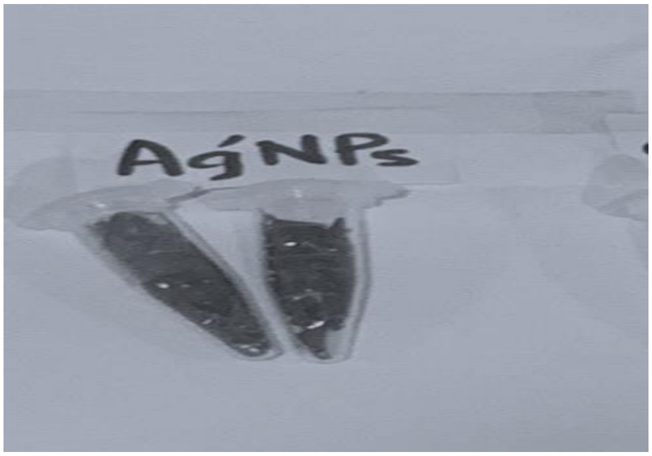
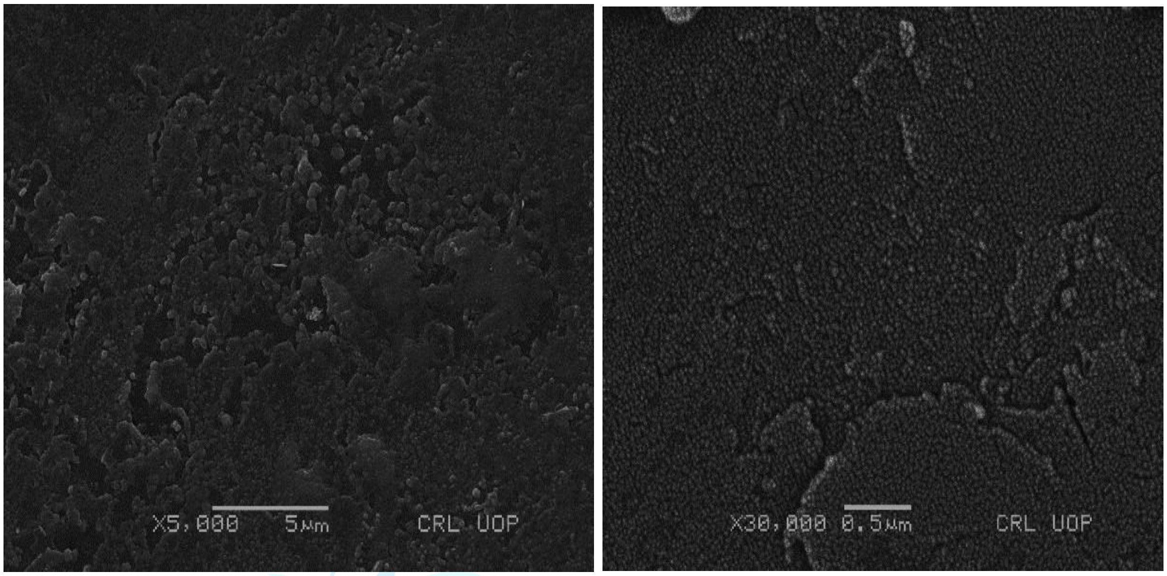


Fig. 4.3. Purified Pellets Chitosan Mediated Silver Nanoparticles

Characterization of Chitosan mediated AgNPs
Scanning Electron Microscope
Using SEM techniques at various magnifications, the stability, shape, and surface area of AgNPs will be investigate the produced AgNPs are primarily spherical with some degree of agglomeration, the effective synthesis is confirmed by the homogeneous distribution of nanoparticles with diameters in the nanometer range. SEM analysis of the produced silver nanoparticles (AgNPs)

verified the generation of mostly spherical nanoparticles with minimal agglomeration, which could be caused by high surface energy or sample preparation. Successful synthesis and stability are indicated by the well-dispersed, nanometer-sized particles. High surface energy and interactions during sample preparation could be the cause of the slight clustering that was seen, the results of SEM are shown in Figure. 4.4 (a,b).



(a) (b)

Fig, 4.4(a). Scanning Electron Micrograph of chitosan mediated AgNPs
(b). Scanning Electron Micrograph of chitosan mediated AgNPs

Zeta Sizer Analysis of AgNPs

Using the Malvern Zetasizer Nano ZS equipment, The produced nanoparticles showed a moderately polydisperse system with a Z-average of size 394.1 Nano meter and a polydispersity index (PDI) of 0.457, With an intensitybased mean size of 273.3 nm, the majority of the particle population indicated the existence of both tiny and aggregated nanoparticles. The measurements' dependability was validated by the correlation fit parameters, which included an intercept of 0.9819 and a low fit error of 0.004624.the average chitosan mediated particles size was analyzed to be 70.01 nm.

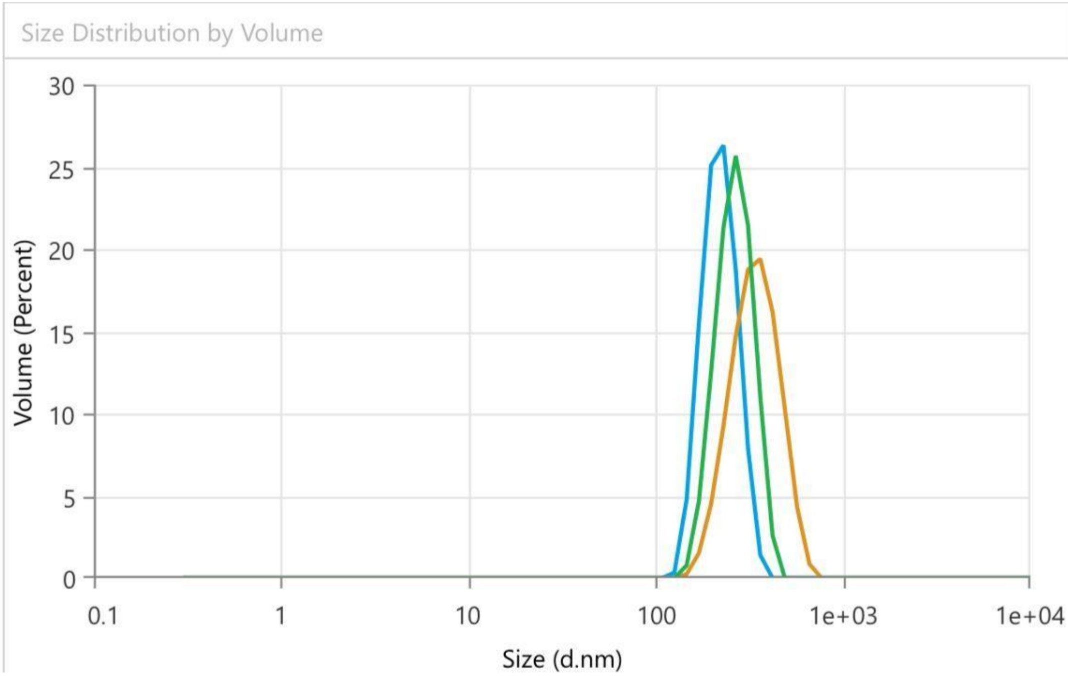


Fig. 4.5. (a) Zeta Sizer Grapic Result of AgNPs

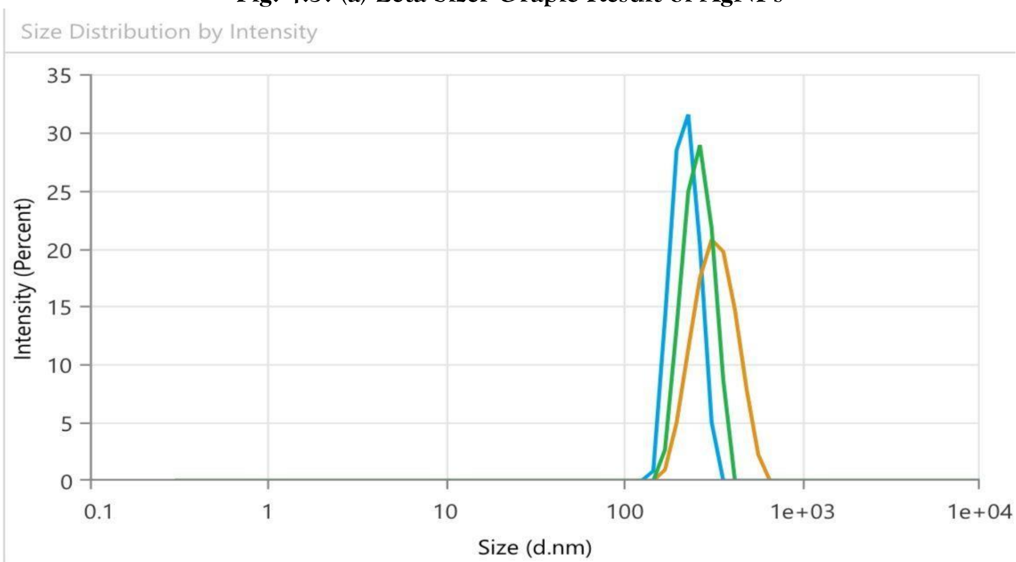


Fig. 4.5. (b) Zeta Sizer Grapic Result of AgNPs

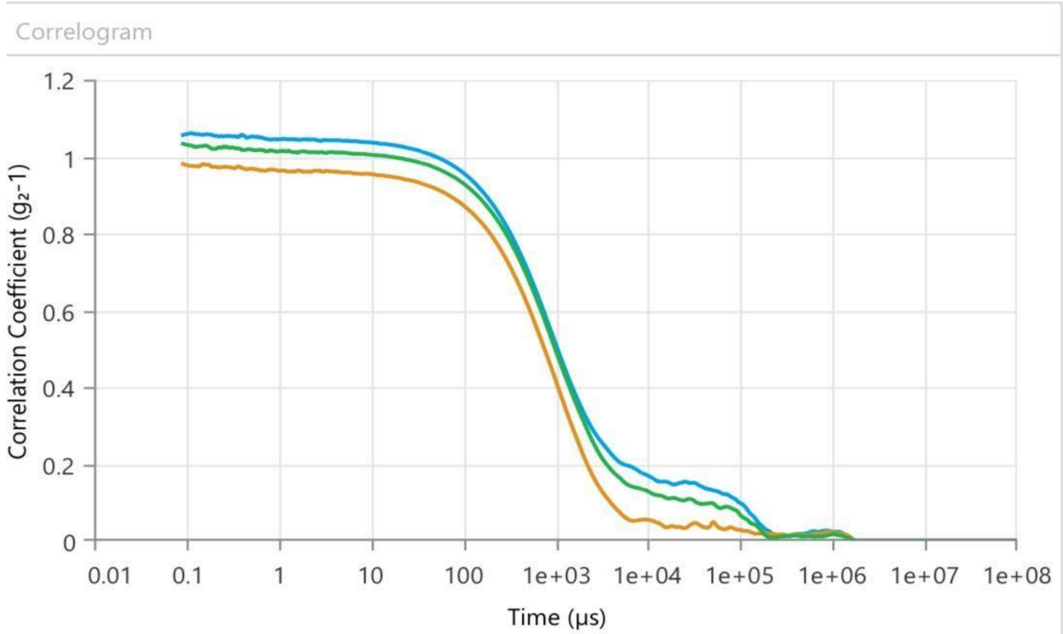


Fig. 4.6. (a) Zeta Sizer Grapic Result of AgNPs

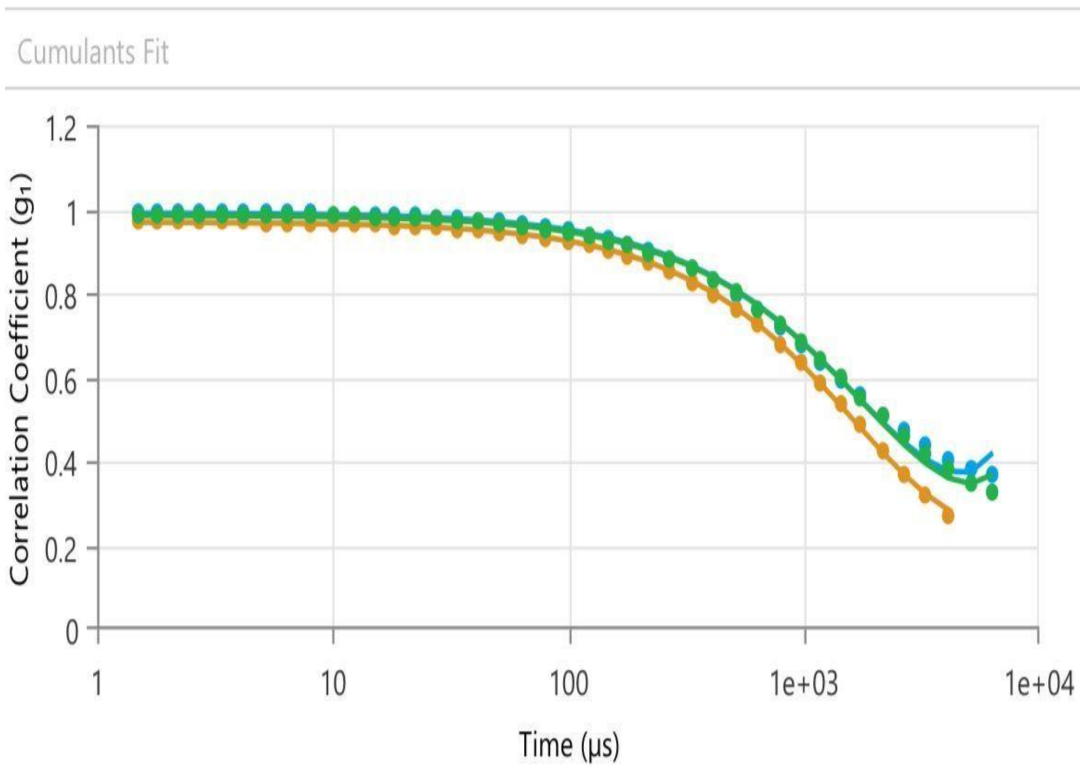


Fig. 4.6 (b) Zeta Sizer Analysis of chitosan mediated AgNPs

Dispersive Energy-Based X-Ray Spectroscopy

The composition of the elemental produced chitosan-mediated silver nanoparticles (AgNPs) was validated by the EDX analysis. The effective synthesis of AgNPs is evident from the presence of a significant silver (Ag) signal with a weight percentage of 46.59% and an atomic percentage of 12.51%. Furthermore, components that correlate to the chitosan capping and stabilizing agent utilized in the synthesis are seen, including carbon

(C), nitrogen (N), and oxygen (O). Remaining ions from the reaction medium may be the cause of the potassium (K) presence. The result of EDX are display in given Table 4.1 and Figure. 4.7.

Table 4.1. Of AgNPs EDX Result

Element	Weight %
C	17.33
N	5.62
O	10.77
K	19.68
Ag	46.59
Totals	**100.00**

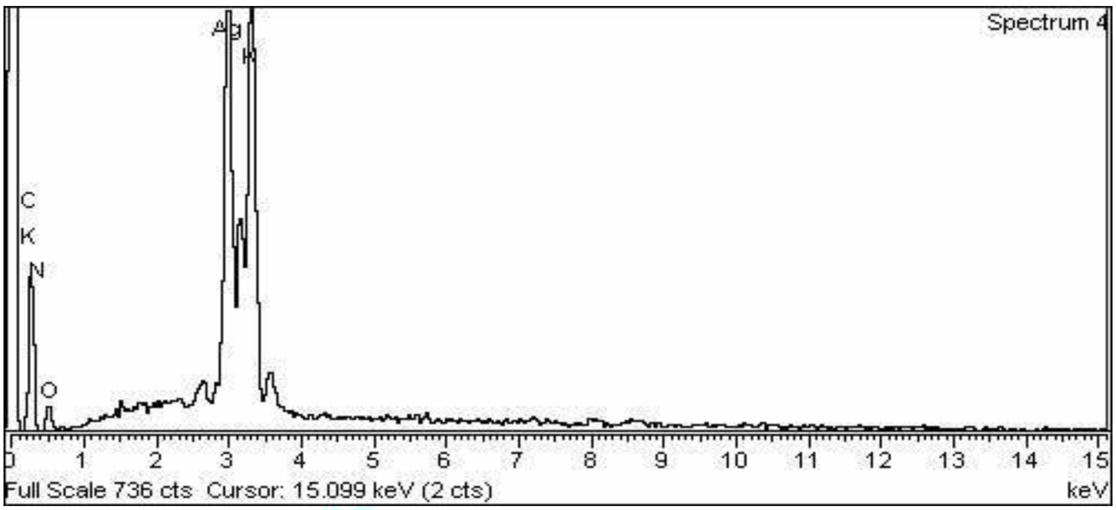


Fig. 4.7 Chitosan mediated AgNPs EDX analysis

X-Ray Diffraction Measurements

The amorphous character of the chitosan matrix is shown by the wide diffraction peak about 23.99° (2θ) in the XRD study of chitosan-mediated AgNPs. The lack of high and sharp peaks indicates that the silver nanoparticles are stable and well-embedded in the chitosan polymer. Despite the absence of identifiable crystalline silver peaks, which are usually located at 2θ values of roughly , 38°, 44°, 64°, and 77° (corresponding to the (111), (200), (220), and also (311) planes of face-centered cubic (fcc) silver), this pattern validates the formation of highly dispersed or ultra-small AgNPs, potentially in a semicrystalline or amorphous phase as a result of chitosan capping. Key Findings of the XRD Analysis: The amorphous form of chitosan is shown by the broad peak at 23.99°. The lack of distinct silver peaks indicates well-dispersed AgNPs or tiny particle sizes. The outcome validates that the chitosan polymer successfully synthesized and capped/stabilized AgNPs. The results are shown in Fig.4.8.

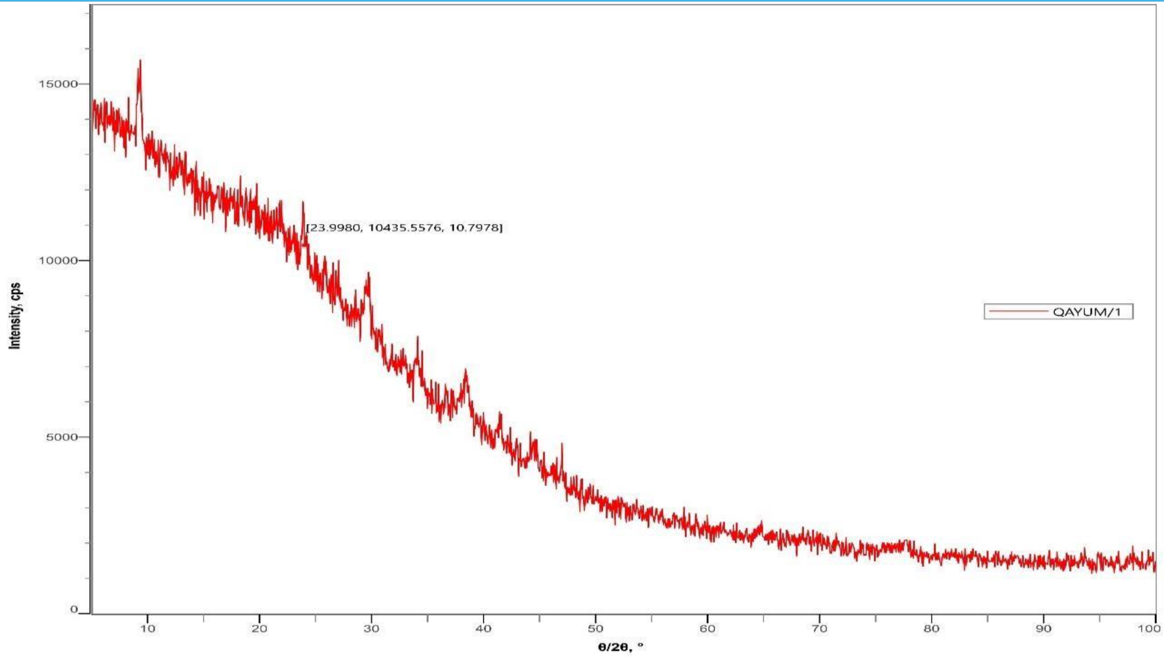


Fig. 4.8. XRD Analysis of chitosan mediated AgNPs

Fourier-Transform Infrared Spectroscopy

Chitosan-mediated AgNPs' FT-IR spectra display distinctive absorption peaks that point to the existence of functional groups that stabilize and reduce the silver nanoparticles. The existence of chitosan and its function in capping AgNPs are suggested by a peak seen at around 1371.39 cm^{-1}, which is corresponds to the Carbon–Hydrogen binding vibration in methyl groups. The Carbon–Oxygen stretching vibrations and Carbon–Hydrogen bending are linked to peaks about 1016.49 cm^{-1} and 798.53 cm^{-1}, respectively, which further demonstrate how the chitosan functional groups interact with AgNPs. Although less pronounced here, the wide range of 3200–3400 cm^{-1} typically corresponds to O–H and N–H stretching, suggesting the role of hydroxyl and amine groups in stabilizing nanoparticles. The results are shown in in Fig. 4.9.

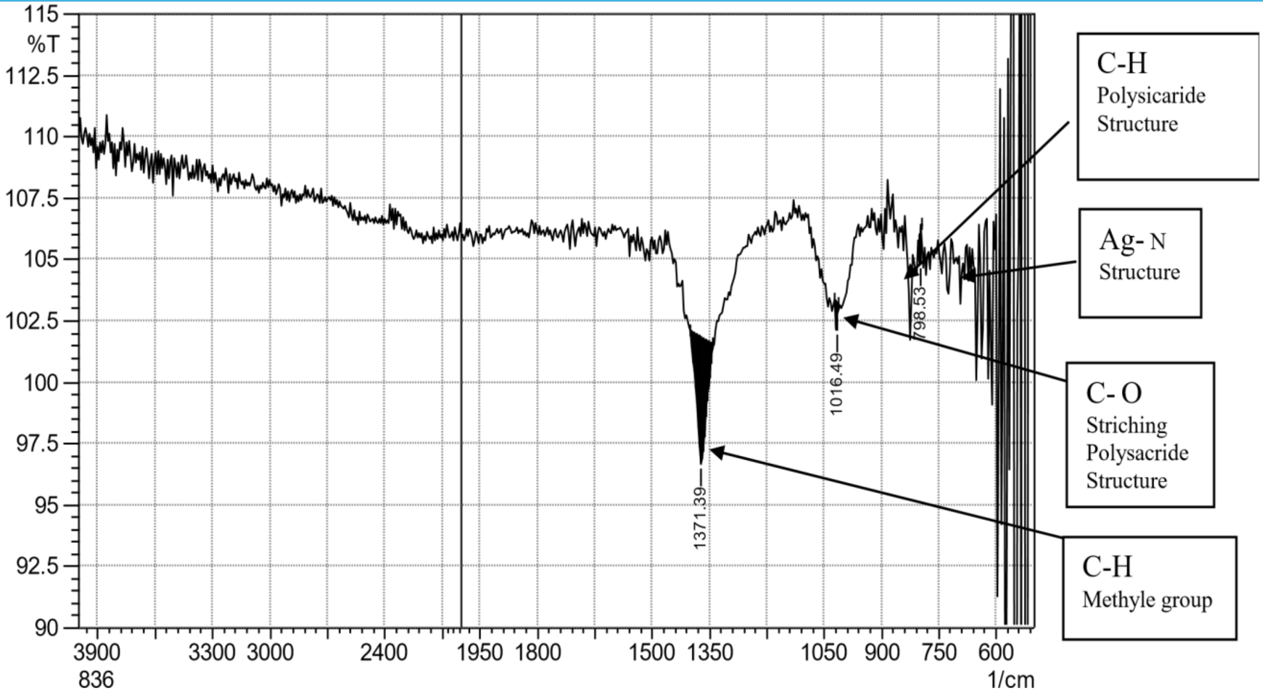


Fig. 4.9. FT-IR Analysis of chitosan mediated AgNPs

INVIVO PHARMACOLOGICAL ACTIVITIES

Anti-inflammatory Activity from AgNPs (Carrageenan-induced paw edema)

It was analyzed that for the anti-inflammatory test evaluation, the provided samples of "naturally fabricated AgNPs" were administered to albino mice at the dose range of 100 mg/kg body weight of the mice i.p. in carrageenan- induced paw edema model/ test.

Details are given below:

Table 4.2. Comparative Anti-Inflammatory Activity Chitosan Mediated AgNPs and Chitosan using mice models

S. No.	Groups	Treatment	Dose /kg 100 μg/mL	Percent Inhibition (%)		
				After 1 hour	After 2 hours	After 3 hours
1	I	Saline (Control)	100 μg/mL	0	0	0
2	II	Chitosan	100 μg/mL	**10.37**	**16.32**	**33.55**
3	III	AgNPs				

		100 µg/mL	27.58	58.91	73.84

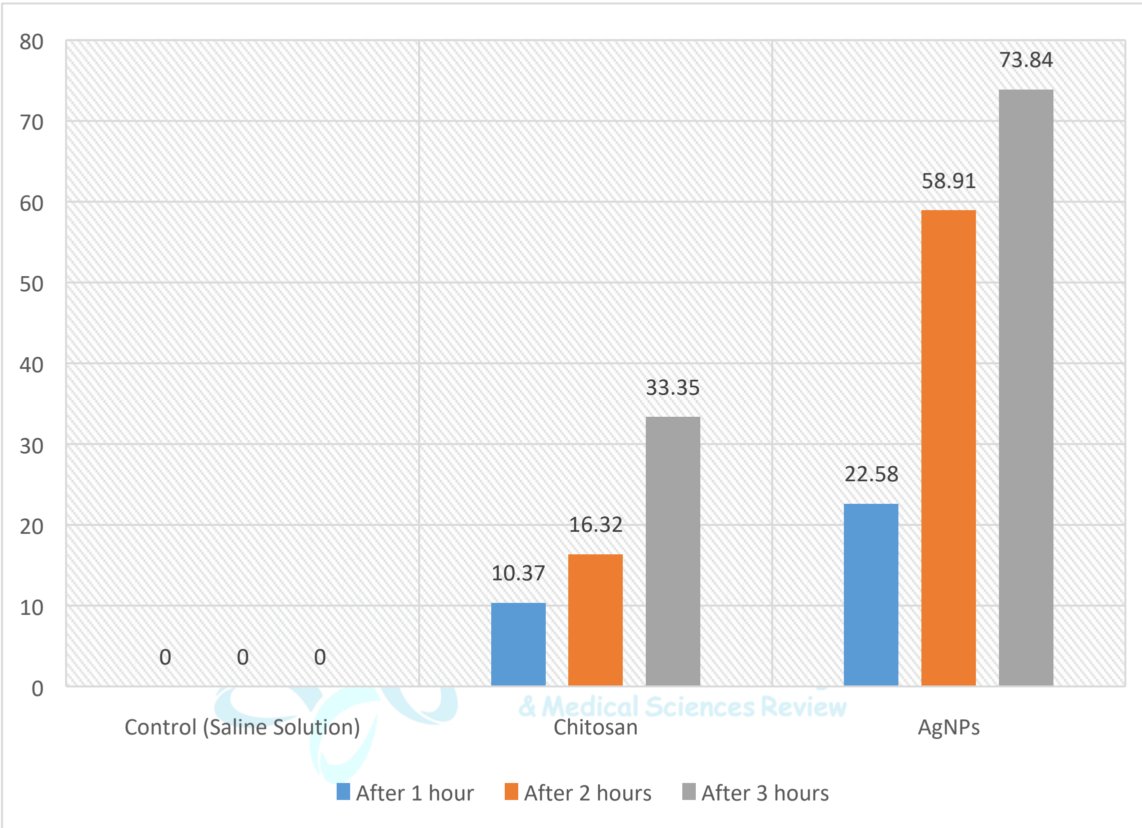


Fig. 4.10 Comparative Anti-Inflammatory Activity Chitosan Mediated AgNPs and Chitosan using mice models

Anti-pyretic Activity of AgNPs

For the antipyretic test evaluation, the provided samples of "Naturally Fabricated AgNPs" were administered to albino mice at the dose rang of 100 mg/kg their body weight of mice i.p. in yeast induced pyrexia model/ test. Details are given below:

Table 4.3. Comparative Anti-Antipyretic Activity Chitosan Mediated AgNPs and Chitosan using mice models

S. No.	Groups	Treatment	Dose /kg 100 µg/mL	Percent Inhibition (%)		
				After 1 hour	After 2 hours	After 3 hours
1	I	Saline		0	0	0

		(Control)	100 µg/ mL			
2	II	Chitosan	100 µg/ mL	12.16	18.58	30.19
3	III	AgNPs	100 µg/ mL	35.25	53.28	76.98

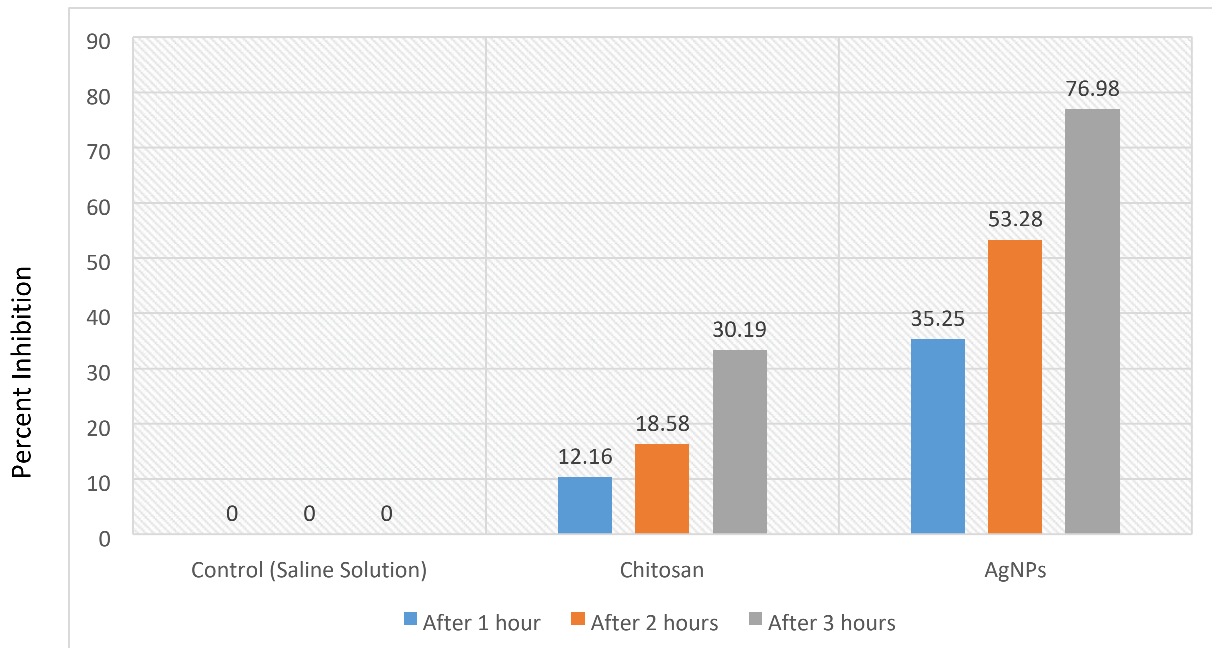


Fig. 4.11 Comparative Anti-Pyretic Activity Chitosan Mediated AgNPs and Chitosan using mice models

DISCUSSION

In the domain of technology and science, Sustainable nanotechnology has proven to be successful in solving issues across a range of domain , know a day the scientist focus on nano-particles in the field of medicine, Chitosan have the ability as reducing agent, to prepared effective and stabilized AgNPs, (Beyene *et al.*, 2017). In this research we prepared chitosan mediated AgNPs alternate physical and chemical methods, Chitosan and other medicinal polysaccharides have recently been shown to function as potential bioreducing agents that may be used to create efficient AgNPs. This technique has no side effect and harmful. (Ramalingam *et al.*, 2014). Previously are the AgNPs are characterized by spectoscipic method this synthesized chitosan AgNPs are characterized by spectroscopic methods the AgNPs are find out range 7-70 nm ,stability, shape, and surface area of AgNPs will be investigate The produced AgNPs are primarily spherical with some degree of agglomeration, according to SEM examination. The effective synthesis is confirmed by the homogeneous distribution of nanoparticles with diameters in the nanometer range, produced nanoparticles showed a moderately polydisperse system with a Zeta sized of 394.1 nm and a polydispersity index (PDI) of 0.457, With an

intensity-based mean size of 273.3 nm,the elemental makeup of the produced chitosan-mediated AgNPs was validated by the EDX analysis.
Shinde *et al,* (2021) The effective preparation of AgNPs is evident from presence of a significant silver (Ag) signal with a weight percentage of 46.59% and an atomic percentage of 12.51%.the amorphous character of the chitosan matrix is shown by the wide diffraction peak about 23.99° (2θ) in the XRD study of chitosan-mediated AgNPs. The lack of high and sharp peaks indicates that the silver nanoparticles are stable and wellembedded in the chitosan polymer, chitosan-mediated AgNPs' FT-IR spectra display distinctive absorption peaks that point to the existence of functional groups that stabilize and reduce the silver nanoparticles. Shinde *et al.* (2023) The existence of chitosan and its function in capping AgNPs are suggested by a peak seen at range 1373 cm^{-1}, which corresponds to the C–N stretching of amine groups. The C–O stretching vibrations and C–
H bending are linked to peaks about 1016 cm^{-1} and 724 cm^{-1}, respectively, from the previous biological study of AgNPs is According to Center of Disease Control and Prevention, a big portion of world population is afflicted with minor to major diseases. The most common affliction that irritates the body is inflammation and pyrexia. There are numerous allopathic medications that target either the infection or the symptom of pyrexia. The difficulty in controlling and treating vectors has increased due to drug resistance. However, due to limited access to synthetic pharmaceuticals, many marginalized individuals are forced to cure numerous diseases with locally available natural resources such as plants, fungi and beneficial bacteria. Advances in phytochemical research and nanotechnology are also capturing the interest of researchers looking to identify new anti-pyretic and anti-inflammatory agents from this research we were check this chitosan mediated AgNPs for biological activity they are shown a good effect against anti-inflammatory and anti-pyretic activity in mice models. This study lacked a consistent distribution of particle sizes and did not employ sophisticated imaging techniques such as TEM. The long-term stability of AgNPs and stabilizing chemicals were not thoroughly evaluated. Brief observation periods and delivery of a single dosage. No toxicological or chronic investigations were carried out, nor were the mechanisms behind the antipyretic and anti-inflammatory actions investigated. (Chaudhary*et al.*, 2023).

CONCLUSION & RECOMMENDATIONS

CONCLUSION

Considering the findings of the present investigation, it was determined that chitosan has ability as reducing agent and prepared a strong, stable, shape, and the surface area of AgNPs, The produced AgNPs mostly spherical High surface energy and most effectively active nanoparticles range 70-100, the chitosan mediated AgNPs was show as good in vivo anti-inflammatory and antipyretic activity, average highest % anti-inflammatory activity (73.84%) and / antipyretic activity (78.98%) respectively.

RECOMMENDATIONS

The giving recommendations are offered for further research;

1.Investigation of various bio-reduce chitosan that can be precisely used to produce AgNPs.
2.The active AgNPs can be used to manufacture new anti-microbial and anti-toxicity which could be used as community medicine and for further research.
3.Investigation of AgNPs that may find application in the fields of engineering and agriculture for the development of Nano devices, biosensors, bio-catalysts, and diagnostic studies.

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