ORIGINAL ARTICLE

Biosynthesis of Silver Nanoparticles Using *Abutilon indicum* (Link): An Investigation of Anti-inflammatory and Antioxidant Potential against Carrageen Induced Paw Edema in Rats

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Abstract

Aim: In the present study, the synthesis of silver nanoparticle from ethanolic extraction of *Abutilon indicum* (Link) and its anti-inflammatory & antioxidant properties were studied. The bioactive compounds from *Abutilon indicum* were identified by gas chromatography and mass spectroscopy. The AgNPs synthesis was done using the ethanolic solution of *A. indicum* extract and silver nitrate. **Materials and Methods:** The AgNPs were characterized using ultraviolet (UV)-spectroscopic analysis, high-resolution transmission electron microscopy (HRTEM), and Fourier transform infrared spectroscopy (FTIR) analysis. The anti-inflammatory and antioxidants assays were followed by the standard methods. The mice bioassay study was conducted using doses of 50, 100, 150, and 200 mg/kg bw. **Results:** The presence of bioactive compounds from *A. indicum* is dodecanoic acid (8.846), octadecanoic acid (9.608), nonanoic acid (12.258), N-hexadecanoic acid (14.222), ethanol, 2-bromo (16.985), dibutyl phthalate (17.729), phytol (19.671), and diisooctyl phthalate (23.272). UV-visible spectral analysis shows a maximum absorption peak at 421.00 nm. The FTIR spectra of AgNPs exhibited prominent peaks represents such as alcohols, phenols, alkynes, aromatics, aldehydes, alkenes, aromatics, aliphatic amines, 1° and 2° amines, and alkyl halides. The HRTEM analysis of the synthesized AgNPs clearly showed the clustered and irregular shapes, mostly aggregated and having the size of 20 nm. The maximum inhibition of edema 39.49% was found in ethanolic extraction of *A. indicum* (200 mg/kg bw) and AgNPs of *A. indicum* (100 mg/kg bw) showed 55.98% at the end of 3 h. A significant increase in the activities of superoxide dismutase, catalase, glutathione peroxidase, vitamin-C and vitamin-E was observed in tissue of inflammation in rats on treatment with 100 mg/kg body weight of *A. indicum* extract and compared with standard and control groups. **Conclusion:** This study indicates the AgNPs synthesized *A. indicum* possesses anti-inflammatory and antioxidant potential which may be used for therapeutic purposes mainly in the prevention of oxidative damage that occurs during inflammation.

Key words: *Abutilon indicum,* anti-inflammatory, antioxidants, silver nanoparticles

INTRODUCTION

Despite the progress made in medical research for the past decades, the treatment of many serious diseases is still problematic. Chronic inflammatory diseases remain one of the world's major health problems. Inflammation is the immunological response of living tissues to injury. It involves a complex array of enzyme activation, mediator release and extravasations of fluid, cell migration, tissue breakdown, and repair. The current pharmacological management of inflammation is mainly by two groups of drugs, the steroidal anti-inflammatory drugs and the nonsteroidal anti-inflammatory agents. However, these conventional drugs are associated with numerous side effects that have compelled the need for identification of alternative

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substances that can resolve inflammation in a way that is homeostatic, modulatory, efficient, and well-tolerated by the body.[1] The use of non-steroidal anti-inflammatory drugs and analgesic agents has not been successful in all cases because of its adverse effects such as gastric lesions. One such alternative rationale for treatment of inflammatory disorders is phytomedicine. The natural compounds from plants have aided in the synthesis of new anti-inflammatory drugs with higher pharmaceutical value.[2]

The use of anti-inflammatory agents is helpful in the therapeutic treatment of pathologies. The medicinal plants are widely used in folk medicine of many countries to treat different inflammatory conditions and, in particular, skin inflammations. However, many of the plants in use, the real efficacy, and the relevant active principles are unknown. Consequently, experimental studies aimed at demonstrating the pharmacological properties of these plants and identifying the relevant active principles are needed.[3]

Silver nanoparticles (AgNPs) own unique properties which found many applications such as antimicrobial, anticancer, larvicidal, catalytic, and wound healing activities. Biogenic synthesis of AgNPs using plants extracts are gaining drive to their pharmacological and other potential applications. Plant extracts contain phytochemicals which aid in the reduction of the silver ions.[4] The added advantage of using plants is that the alkaloids, flavonoids, tannin, etc. This also acts as capping agents, thereby conferring the AgNPs with additional pharmacological properties.

Abutilon indicum belonging to family *Malvaceae* is distributed throughout all tropical zones. *A. indicum* is reported to be used to treat ulcers, headaches, gonorrhea, bladder infection, inflammation, hepatic, and pulmonary disorders. There are several reports proved that this plant also used as demulcent, aphrodisiac, laxative, diuretic and sedative (leaves), diuretic; laxative, expectorant, and demulcent.[5] The leaves can also be used to treat ulcers, headaches, gonorrhea, and bladder infection.[6] Such plants root, bark, flowers, leaves, and seeds are very much used in Siddha medicines. The leaves are also used for pile complaints.[7] So far not adequate characterization of its analgesic and anti-inflammatory activity has not been yet confirmed. Hence, this study shows the synthesis of AgNPs from ethanolic extraction of *A. indicum* and it is anti-inflammatory and antioxidant properties of carrageenan-induced paw edema in inflammatory rats.

Collection and authentication of experimental plant

Fresh healthy *A. indicum* was collected from Herbal Garden, AMET University campus Chennai and authenticated by professionals in Department of Botany, St. Joseph's College, and Tiruchirappalli. According to Mukerjee,[8] the voucher specimen was deposited at the rapinat herbarium and the voucher number is JS001.

Preparation of extraction

The coarse powdered plant material was extracted with ethanol by using soxhlet apparatus. The solvent were removed under reduced pressure to get crude extract. Standard methods were used for preliminary phytochemical screening of the extract, which was performed to know the phytoconstituents in the extract.[9]

Gas chromatography-mass spectrometry (GC-MS) analysis

GC-MS analysis of the sample was performed using a Shimadzu GCMS-QP2010 gas chromatograph mass spectrometer interfaced with a Turbo Mass quadrupole mass spectrometer, fitted with an Rtx-5 fused silica capillary column (30 mm × 0.25 mm, with 1 Cm film thickness). The oven temperature was programmed from 100°C to 320°C at 100°C/min and a hold for 10 min. Helium was used as carrier gas at flow 1.0 mL/min. The injector temperature was 250°C, injection size 1 µL neat, with split ratio 1:10. The interface and MS ion source were maintained at 320°C and 200°C, respectively, and the mass spectra were taken at 70 eV with a mass scan range of 40-700 atomic mass unit.

Identification of compounds

Interpretation of mass spectrum of GC-MS was conducted using the mass spectral database of National Institute of Standard and Technology (NIST) having more than 62,000 patterns. The spectrum of the unknown component was compared with the spectrum of the known components stored in the NIST library. The name, molecular weight, and structure of the components of the test materials were ascertained.

Preparation of 1 mm silver nitrate ($AgNO_3$) aqueous solution

An accurately weighed 0.017 g of $AgNO_3$ was dissolved in 100 mL of double-distilled water and stored in amber color bottle for further use.

Synthesis of silver nanoparticle from ethanolic leaf extraction of *A. indicum*

The synthesis of AgNP was performed by Vijayaraj *et al.*[10] 5 mL of the ethanolic leaf extract of *A. indicum* was taken in the conical flask separately and placed on a magnetic stirrer with hot plate. To this 50 mL of 1 mM $AgNO_3$ solution was added dropwise with constant stirring of 120 rpm at 50-60°C. The color change of the solution was checked periodically. The color change of the medium from colorless to brown after 5 h was observed which indicated the formation of

AgNPs. It showed that aqueous silver ions could be reduced by the ethanolic extract of *A. indicum* to generate extremely stable AgNPs.

Characterization techniques

Ultraviolet-visible (UV-VIS) spectroscopy

The AgNPs were characterized in a Shimadzu-1800 UV-VIS spectrophotometer. The optical properties (absorbance) of the sample were evaluated at the wavelength range of 300-600 nm. The double-distilled water used as a blank reference.

Scanning electron microscope (SEM)

Thin films of the sample were prepared on a carbon coated copper grid by dropping a very small amount of the sample on the grid. Extra solution was removed using a blotting paper, and then the films on the SEM grid were allowed to dry by putting it under a mercury lamp for 5 min.

Fourier transform infrared spectroscopy (FTIR)

To remove any free biomass residue or compound that is not the capping ligand of the nanoparticles, after complete reduction, AgNPs were concentrated by repeated centrifugation (3 times) of the reaction mixture at 15,000 rpm for 20 min. The supernatant was replaced by distilled water each time. Thereafter, the purified suspension was freeze-dried to obtain dried powder. Finally, the dried nanoparticles were analyzed by ALPHA FTIR spectrometer (from Bruker, Germany) for the detection of different functional groups by showing peaks from the region of 4000-500 cm^{-1}.

Animals

Wistar rats 7-8 week old, weighing 150-200 g were used for the present study. To maintain the animal house under the standard condition of temperature (24 ± 2°C) and relative humidity (30-70) with a 12:12 light:dark cycle. The animals were fed with stranded pellet diet and water. The animal handling was performed according to good laboratory practice. Ethical clearance was obtained from the Institutional Animal Ethical Committee (CPCSEA/265/2015) and conducted according to Indian National Science Academy guidelines for the use and care of experiments.

Acute toxicity study

Animals were randomly allotted in five groups (each group contain six mice). The ethanol extract was administered orally at doses of 50, 100, 200, and 300 mg/kg of body weight. The control group received only the normal saline (10 mL/kg b.w). The animals were observed during the first 2 h for toxic signs, and then mortality was recorded for each group at 24, 48, and 72 h after dose administration.

Animal grouping

Animals were divided into five groups (six animals in each),

- Group 1: Normal control (normal saline)
- Group 2: Negative control (carrageenan, 1%)
- Group 3: Positive control (indomethacin - 10 mg/kg)
- Group 4: Ethanolic extract of *A. indicum* (200 mg/kg)
- Group 5: AgNPs synthesized *A. indicum* (100 mg/kg).

Evaluation of anti-inflammatory activity

Anti-inflammatory activity was tested on extract of *A. indicum* against carrageenan-induced paw edema in rats. The reductions of paw edema of rats are compared with the standard drug, i.e., indomethacin.

$$\text{Percentage inhibition } (\%) = \frac{\text{Control}^* - \text{Test}^*}{\text{Control}^*} \times 100$$

*Increase in paw volume in 3 h.

Preparation of tissue homogenate

The tissue samples were homogenized in a solution containing 5% trichloroacetic acid and 5 mM ethylenediaminetetraacetic acid at 4°C and centrifuged for 10 min at 15,000 g in 4°C.[11]

Estimation of antioxidants

Enzymatic antioxidants

The estimation of enzymatic antioxidants superoxide dismutase (SOD), catalase (CAT), glutathione (GSH) peroxidase (GPx), and GSH was measured. The activity of SOD was assayed by the method of Kakkar *et al.*,[12] CAT was estimated by the method of Sinha,[13] GPx was measured by the method described by Rotruck *et al.*[14]

Nonenzymatic antioxidants

The estimation of nonenzymatic antioxidants vitamins E, vitamin C and GSH was estimated. Vitamins C by the method of Omaye *et al.*,[15] vitamin E was estimated by the method of Baker *et al.*,[16] and GSH was measured by the method of Ellman.[17]

Statistical analysis

All results are presented as mean ± standard error of mean. Data were analyzed by the Student's *t*-test. Groups for the pair of observations depended on each other. Results were considered statistically at $P < 0.05$.

RESULTS

Photochemical analysis

The preliminary phytochemical screening results of *A. indicum* showed [Table 1] various bioactive secondary metabolites constituents such as alkaloids (0.42%), flavonoids (0.62), saponins (4.5%), tannins (1.0%), terpenoids (0.20%), carbohydrates (0.16), and protein (2.5).

GC-MS analysis of *A. indicum*

The bioactive compounds present in the *A. indicum* extract was identified by GC analysis. In total, eight compounds identified from ethanolic extract of the *A. indicum* such as dodecanoic acid (8.846), octadecanoic acid (9.608), nonanoic acid (12.258), N-hexadecanoic acid (14.222), ethanol, 2-bromo (16.985), dibutyl phthalate (17.729), phytol (19.671), and diisooctyl phthalate (23.272). The GC-MS extracts of *A. indicum* of these compounds are of chromatogram of ethanol extracts of *A. indicum* is shown in Figure 1.

Characterization technique

UV-VIS spectrophotometer

The reduction of silver is confirmed in the samples by visual observation. The sample exhibited dark brown. This color variation may be attributed to excitation of surface plasmon vibration in AgNPs. After 24 h incubation in dark room condition, the light colored reaction mixtures turned into dark brown for indicating AgNP formation [Figure 2]. The surface plasmon resonance (SPR) of AgNPs produced a peak at 420 nm [Figure 3], which suggests the dispersal of AgNPs.

FTIR analysis

FTIR analysis shows [Figure 4] the transmittance at 3415.93, 3240.41, 3128.54, 2301.08, 1666.50, 1598.99, 1292.31, 1211.30, 775.38, 501.28 cm^{-1} which indicates the functional group of the plant component involves in the reduction and stabilization of AgNPs. The transmittance attributes O–H stretch, H–bonded,–C≡C–H: C–H stretch, C–H stretch, H–C=O: C–H stretch,–C=C– stretch, C–C stretch (in–ring),C–H wag (–CH_2 X),C–N stretch, N–H wag, and C–Br stretch reveal that the water soluble heterocyclic components, alcohols, phenols, alkynes (terminal), aromatics, aldehydes, alkenes, aromatics, alkyl halides, aliphatic amines, 1° and 2°

Table 1: % of phytoconstituents in ethanolic extraction *A. indicum*

Phytoconstituents	Percentage of phytoconstituents in *A. indicum*
Alkaloids	0.42
Flavonoids	0.62
Saponins	4.5
Tannins	1.0
Terpenoids	0.20
Carbohydrates	0.16
Protein	2.5

A. indicum: Abutilon indicum

Table 2: FTIR analysis

Frequency (cm^{-1})	Bond	Functional group
3415.93	O–H stretch, H–bonded	Alcohols, phenols
3240.41	–C≡C–H: C–H stretch	Alkynes (terminal)
3128.54	C–H stretch	Aromatics
2301.08	H–C=O: C–H stretch	Aldehydes
1666.50	–C=C– stretch	Alkenes
1598.99	C–C stretch (in–ring)	Aromatics
1292.31	C–H wag (–CH_2 X)	Alkyl halides
1211.30	C–N stretch	aliphatic amines
775.38	N–H wag	1°, 2° amines
501.28	C–Br stretch	Alkyl halides

FTIR: Fourier transform infrared

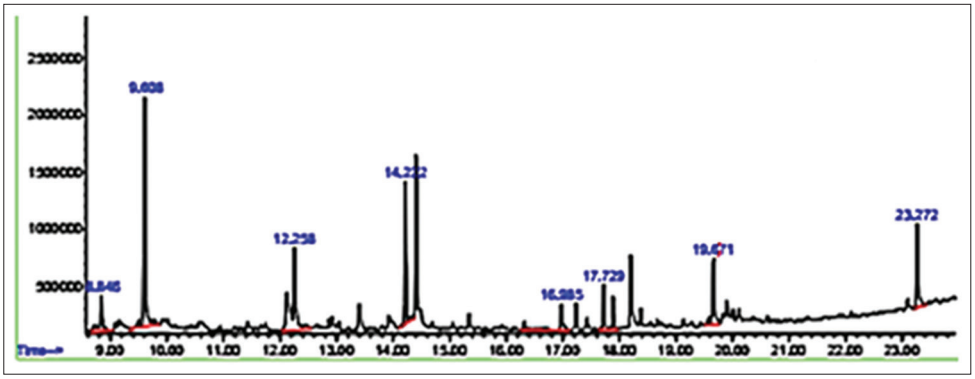

Figure 1: Gas chromatography and mass spectroscopy chromatogram of *Abutilon indicum*

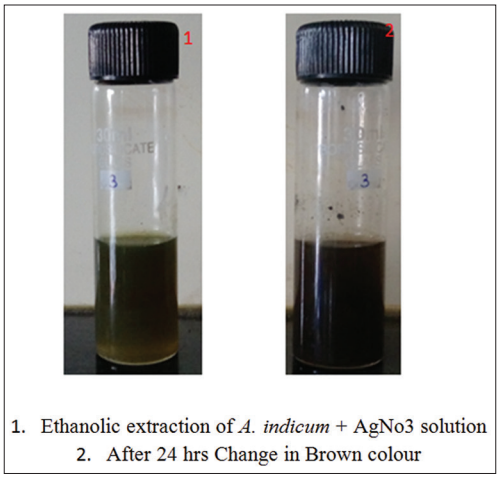

Figure 2: Visible observation of AgNPs from *A. indicum*

amines, alkyl halides present in the extract involved in the reduction of $AgNO_3$ [Table 2].

High resolution transmission electron microscopy (HRTEM) analysis

HRTEM analysis displayed that AgNPs were in two different ranges of size, small particles ranging from 5 to 15 nm and larger particle in the size between 30 and 45 nm. It was further observed that the average size of the particle was found to be 20 nm. It was also observed that the particles were spherical in shape and evenly distributed in the sample. Selected area diffraction graph was interpreted with JCPDS software. Spots inside the circles corresponding to the interplanar distances related to the 111, 200, 220, and 311 planes of the face-centered cubic crystalline structure of silver [Figure 5].

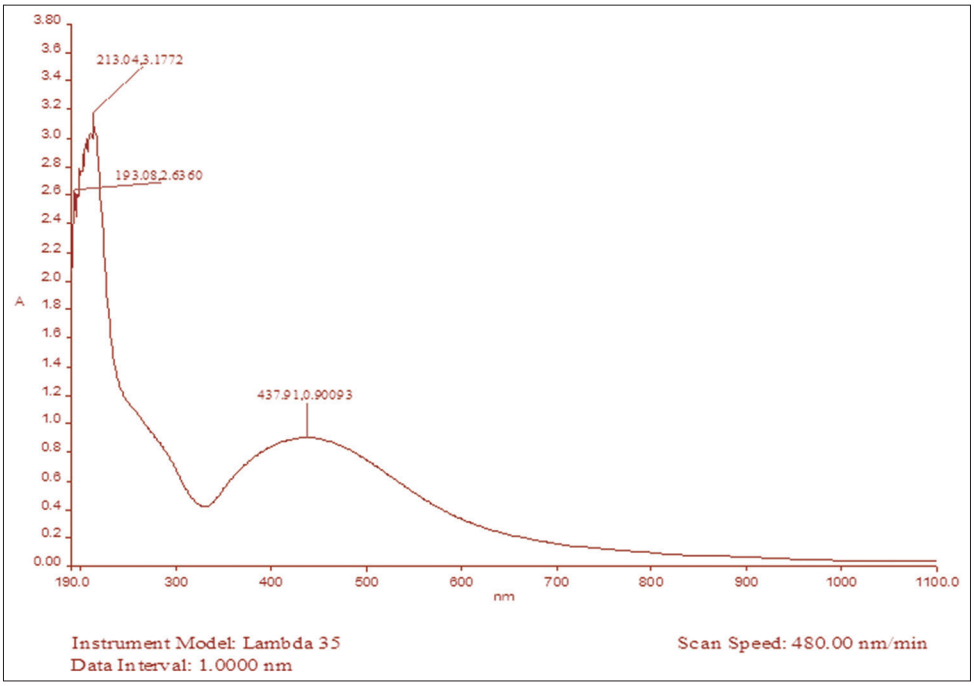

Figure 3: Ultraviolet-visible absorption of silver nanoparticles synthesized *Abutilon indicum*

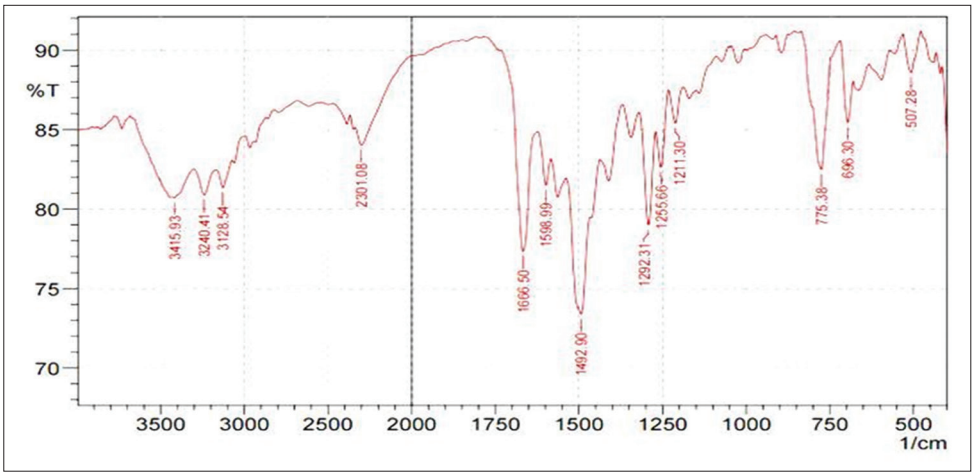

Figure 4: Fourier transform infrared analysis of silver nanoparticles synthesized *Abutilon indicum*

Acute toxicity study

No significant changes were observed in the behavioral or autonomic responses in mice after treatment with different doses of ethanolic leaf extract of *A. indicum*.

Anti-inflammatory activity

In carrageenan-induced paw edema in rats, oral administration of ethanolic leaves extracts and AgNP of *A. indicum* showed inhibition of paw edema at 3 h after carrageenan injection and was compared with standard indomethacin [Table 3]. The ethanolic leaves extract of *A. indicum* (200 mg/kg) and AgNPs of *A. indicum* (100 mg/kg) showed significant inhibition. The anti-inflammatory effect induced by indomethacin progressively increased and reached a maximum of 58.13% after 3 h ($P < 0.05$). AgNPs of *A. indicum* (100 mg/kg) showed better inhibition of paw edema than dose at *A. indicum* 200 mg/kg. Sub-plantar injection of carrageenan in rats showed a time-dependent increase in paw thickness which was observed at 1 h and was maximal at 3 h in the control group [Figure 6].

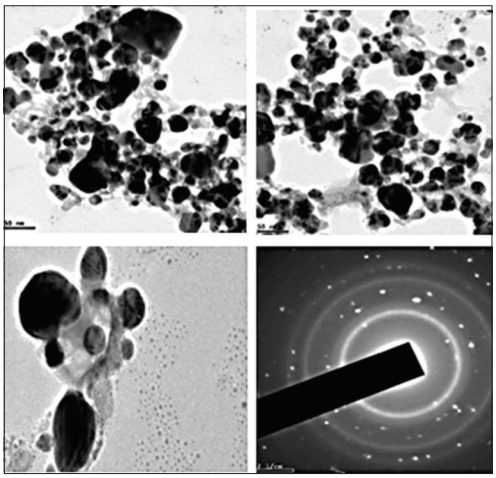

Figure 5: Transmission electron microscopy analysis of silver nanoparticles synthesized *Abutilon indicum*

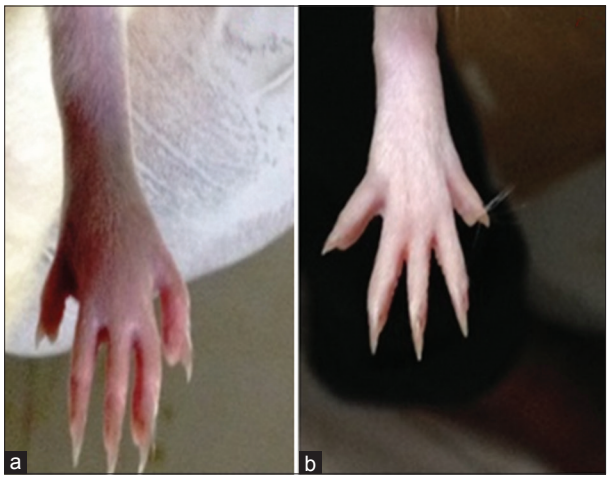

Figure 6: (a) Inflammation induced paw edema, (b) treated paw edema

Enzymatic antioxidants

The levels of enzymatic antioxidant in erythrocyte lysate and inflammatory tissue of control and experimental animals in each group were shown in Table 4. The decreased levels of enzymatic antioxidants status were seen in erythrocyte lysate and inflammatory was observed in carrageenan alone treated rats when compared to control rats. AgNPs of *A. indicum* (100 mg/kg bw) and ethanolic leaves extracts of *A. indicum* (200 mg/kg) treated rats showed significantly normalized the enzymatic antioxidant such SOD, CAT and GPx in carrageenan-treated animals compare to indomethacin (10 mg/kg).

Nonenzymatic antioxidants

The levels of nonenzymatic antioxidants activity in control and experimental animals in each group were shown in Table 5. Increased levels of GSH and decreased levels vitamin E and C were observed in carrageenan alone treated rats when compared to control rats. AgNPs of *A. indicum* (100 mg/kg) and ethanolic leaves extracts of *A. indicum* (200 mg/kg) treated rats showed significantly improved the levels of GSH, vitamin E and vitamin C in carrageenan alone treated rats, compare to standard anti-inflammatory drug indomethacin (10 mg/kg).

DISCUSSION

Natural drugs have been a part of the evolution of human, health care for thousands of years. Nowadays, nearly 88% of the global populations turn to plant-derived medicines as their first line of defense for maintaining health and combating diseases. So far 119 metabolites derived from plants are used globally as drugs, 15% of all angiosperms have been investigated chemically and of that 74% of pharmacologically active plant-derived components were discovered.[18] Plants are rich in a wide variety of secondary metabolites such as tannins, terpenoids, alkaloids, and flavonoids which have been found *in vitro* to have medicinal properties. This study reveals that ethanolic extract of *A. indicum* exhibited the presence of alkaloids, terpenoids, flavonoids, phenol, tannins, phytoserol, carbohydrates, and saponins. In phytochemical analysis the major compounds of alkaloids, phenols, tannins, etc., are rich in medicine and constitute most of the valuable drugs. They have physiological effect on animals.[19]

The pharmacological studies have accepted the value of medicinal plants as potential source of bioactive compounds.[20] The medicinal plants are sources of bioactive compounds and play a dominant role in the maintenance of human health. Green plants are reservoir of effective chemotherapeutants; these are non-phytotoxic, more systemic and easily biodegradable.[21] The identification of bioactive compounds in recent years has been increasingly

Table 3: Anti-inflammatory activity of *A. indicum*

Treatment (mg/kg)	Mean increase in paw volume				Percentage of paw volume after 3 h
	0 h	1 h	2 h	3 h	
Control	39.63±2.16	85.11±4.15	103±2.33	123.31±9.33	-
A. indicum extract (200 mg/kg)	22.11±2.18*	39.73±4.05*	63.35±4.18*	74.77±3.58*	39.49%
AgNPs synthesized of *A. indicum* (100 mg/kg)	31.37±1.98*	71.37±2.67*	71.16±2.18*	54.41±1.69*	55.98%
Indomethacin (10 mg/kg)	25.71±1.69**	28.43±1.94*	49.11±1.69*	51.75±2.15**	58.13%

Values are expressed mean±SD for 6 animals in each group. Values not sharing a common superscript significantly differ at $P<0.05$.
SD: Standard deviation, *A. indicum: Abutilon indicum*, AgNP: Silver nanoparticles

Table 4: Enzymatic antioxidants activity of *A. indicum*

Groups/Treatment	Erythrocyte lysate			Inflammatory tissue		
	GPX (U/g Hb)	SOD (U/g Hb)	CAT (U/g Hb)	GPx (U/g protein)	SOD (U/g Hb)	CAT (U/g Hb)
Control	15.79±1.10	8.02±0.89	5.90±0.44	5.26±0.48	8.90±0.73	9.50±0.55
Carrageenan alone	08.29±2.87	02.90±0.20	01.10±1.78	4.05±1.12	7.60±2.2	8.90±0.15
Carrageenan+*A. indicum* (200 mg/kg bw)	10.54±1.37*	05.02±0.50*	04.01±0.59*	6.92±0.67*	12.93±1.17*	9.72±1.03*
AgNPs synthesized *A. indicum* (100 mg/kg bw)	13.09±1.20**	06.45±0.28**	05.09±0.47**	6.79±0.63**	14.27±1.47**	13.28±1.20**
Indomethacin (10 mg/kg)	15.02±1.25**	06.77±0.81**	05.89±0.21**	6.98.12±0.16**	15.12±1.45**	15.21±1.23**

Values are expressed mean±SD for 6 animals in each group. Values not sharing a common superscript significantly differ at $P<0.05$.
SD: Standard deviation, *A. indicum: Abutilon indicum*, AgNP: Silver nanoparticles, GPx: Glutathione peroxidase, SOD: Superoxide dismutase, CAT: Catalase

Table 5: Nonenzymatic antioxidant activity of *A. indicum*

Groups/treatment	Nonenzymatic antioxidants		
	GSH (mg/dl)	Vitamin E (mg/dl)	Vitamin C (mg/dl)
Control	27.36±1.81	1.41±0.15	1.64±0.11
Carrageenan alone	15.13±1.22	0.83±0.07	0.89±0.05
A. indicum extract (100 mg/kg bw)	21.76±1.66*	1.16±0.14*	1.31±0.10*
AgNPs synthesized *A. indicum* (100 mg/kg bw)	23.79±1.80**	1.21±0.10**	1.54±0.10**
Indomethacin (10 mg/kg)	22.15±1.23**	1.26±0.18**	1.14±0.28**

Values are expressed as mean±SD. Values not sharing a common superscript significantly differ at $P<0.05$. *A. indicum*: *Abutilon indicum*, AgNP: Silver nanoparticles, GSH: Glutathione

applied for the analysis of medicinal plants using GC-MS, this techniques has proved to be a valuable method for the analysis of polar and non-polar components.

In GC-MS analysis, totally eight compounds identified from the *A. indicum* extract [Figure 1] such as dodecanoic acid,[22] octadecanoic acid,[23] nonanoic acid,[24] n-hexadecanoic acid,[25] ethanol, 2-bromo, dibutyl phthalate,[26] phytol,[27] and diisooctyl phthalate.[28] All these compounds are reported as pharmacological importance and most of the compounds responsible for anti-inflammatory activity.

The development of green processes for the production of nanoparticles is evolving into a significant branch of nanotechnology.[29] Nanotechnology is expected to be the basis of many technological innovations in the 21st century. The synthesis of nanoparticles is a promising research field due to the possible applications for the extension of novel technologies.[30] Biological synthesis process provides a wide range of environmentally acceptable methodology, low cost production and minimum time required. At the same time, the biologically synthesized AgNPs have many applications in the field of medicine and agriculture.[31]

In $AgNO_3$, solution change of color indicates the formation of AgNPs.[32] Silver nitrate is used as reducing agent and silver has distinctive properties such as good conductivity, catalytic and chemical stability. The aqueous silver ions when exposed to herbal extracts were reduced in solution, thereby leading to the formation of silver hydrosol. The synthesis of AgNPs had been confirmed by measuring the UV-VIS spectrum of the reaction media [Figure 3].

In the present findings, the UV-VIS spectrum of AgNPs synthesized from seed of *A. indicum* has strong absorbance peaks at 420 nm [Figure 3], and the broadening of peaks indicated that the particles are polydispersed. Almost all similar results were observed in *Clerodendrum inerme,*[33] *Euphorbia hirta,*[34] and *Achyranthes aspera.*[10] The weak absorption peaks at shorter wavelengths are due to the presence of several organic compounds which interacts with silver ions.[35] AgNPs have free electrons, which give rise to an SPR absorption band due to the combined vibration of electrons of metal nanoparticles in resonance with the light wave.[36]

FTIR is an important tool which enables us to understand the involvement of functional groups in the interactions between metal particles and biomolecules.[37] FTIR gives the information about functional group present in the synthesized AgNPs for understanding their transformation from simple inorganic $AgNO_3$ to elemental silver. Our study suggested that the FTIR analysis confirmed that the bioreduction of silver ions to AgNPs is due to the reduction by capping material of the plant extract. FTIR spectra of the sample are given in Figure 4. The transmittance at 3415.93, 3240.41, 3128.54, 2301.08, 1666.50, 1598.99, 1292.31, 1211.30, 775.38, and 501.28 cm^{-1} which indicates the functional group of the plant component involves in the reduction and stabilization of AgNPs. The transmittance attributes O–H stretch, H–bonded,–C≡C–H: C–H stretch, C–H stretch, H–C=O: C–H stretch, –C=C– stretch, C–C stretch (in–ring), C–H wag (–CH_2 X), C–N stretch, N–H wag, C–Br stretch reveals that the water-soluble heterocyclic components, alcohols, phenols, alkynes (terminal), aromatics, aldehydes, alkenes, aromatics, alkyl halides, aliphatic amines, 1° and 2° amines, and alkyl halides present in the extract involved in the reduction of $AgNO_3$.

The inflammation is a biological complex of vascular tissues in harmful stimulated by pathogens and irritants and has been major health problems in the world.[10] The anti-inflammatory effects can be elicited by a variety of chemical agents and that there is little correlation between their pharmacological activity and chemical structure.[38] Carrageenan-induced hind paw edema is the standard experimental model of inflammation. Carrageenan is the phlogistic agent of choice for testing anti-inflammatory drugs as it is not known to be antigenic and is devoid of apparent systemic effects. Moreover, the experimental model exhibits a high degree of reproducibility.[39] Carrageenan-induced edema is a biphasic response. The first phase is mediated through the release of histamine, serotonin and kinins, whereas the second phase is related to the release of prostaglandin and slow reacting substances which peek at 3 h.[40] This study of anti-inflammatory activity of ethanol extract of *A. indicum* against carrageenan-induced paw edema shows that the extracts have a significant effect on inflammation and markedly reduced the swelling. The percentage reduction in the paw volume in the group of animals treated with *A. indicum* extract (200 mg/kg bw) was 39.49% and AgNPs synthesized *A. indicum* (100 mg/kg bw) was 55.98% at 3 h. It shows that the plant extract have significant ($P < 0.01$; $P < 0.001$) anti-inflammatory effect and the results were compared with indomethacin (10 mg/kg bw) and show percentage paw volume reduction of 58.13%.

Free radicals have long been implicated as mediators of tissue damage in inflammation patients, which are released in large amounts into the surrounding tissue. To neutralize this charge, free radicals try to withdraw an electron from, or donate an electron to, a neighboring molecule. Other antioxidants work against the molecules that form free radicals, destroying them before they can begin the domino effect that leads to oxidative damage. For example, certain enzymes in the body, such as SOD, CAT, GPx and GSH, work with another chemical to transfer free radical into harmless molecules.[41]

Oxidative stress is a condition of reduction in antioxidative enzymes such as SOD, CAT, GPx, and GSH S-transferases.[42] The antioxidant enzymes SOD and CAT play an important role in reducing cellular stress. SOD scavenges the superoxide radical by converting it to hydrogen peroxide and molecular oxygen, while CAT brings about the reduction of hydrogen peroxides and protects higher tissues from the highly reactive hydroxyl radicals.[43] Table 4 shows that the activities of SOD, CAT, and GPx were significantly decreased in tissue of inflammation control rats due to inadequacy of the antioxidant defenses in combating reactive oxygen species-mediated damage. The decreased levels of enzymatic antioxidants status were seen in erythrocyte lysate and inflammatory was observed in carrageenan alone treated rats when compared with control group. Ethanolic extracts of *A. indicum* at a dose of 100 mg/kg bw significantly normalized the enzymatic antioxidant such SOD, CAT, GPx in carrageenan-treated animals.

The decreased activities of CAT and SOD may be a response to increased production of H_2O_2 and O_2 by the autoxidation. These enzymes play an important role in maintaining physiological levels of oxygen and hydrogen peroxide by hastening the dismutation of oxygen radicals and eliminating organic peroxides and hydroperoxides generated from inadvertent exposure to carrageen.[44] Treatment with extract of *A. indicum* increased the activity of these enzymes and may help to control free radicals when compared to inflammation rats. The effect produced by plant extract was comparable with that of standard drug indomethacin.

Vitamin C plays a central role in the antioxidant protective system, protecting all lipids undergoing oxidation and diminishing the number of apoptotic cells[45] and it also regenerates the oxidized vitamin E. Vitamin E, on the other hand, acts as a non-enzymatic antioxidant and reduces chain reactions of lipid peroxidation.[46] Table 5 showed decreased levels of nonenzymatic antioxidant vitamin C and E in inflammation rats when compared to that of control rats. The levels of these antioxidants were significantly increased

in tissue of inflammatory rats by treating with root extract of *A. indicum*. GSH has a multifaceted role in antioxidant defense. It is a direct scavenger of free radicals as well as a cosubstrate for peroxide detoxification by GPxs.

CONCLUSION

The ethanolic leaf extract of *A.indicum* has shown medicinal importance with anti-inflammatory properties. This study opens up a new opportunity of conveniently synthesizing AgNPs using natural products which could be useful in various applications. Our findings could be targeted for the promising potential applications including drug formulation and biomedical applications in future.

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