

Green Synthesis and *In Vivo* Trials of Extract of *Azadirachta indica* leaf and Bark

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ABSTRACT

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This study investigates the pharmacological aspects of *Azadirachta indica* bark and leaf extracts (MtOH, *n*-Hex, DCM, EtAc) both *in vitro* and *in vivo*. *A. indica* has antioxidant qualities in DCM, MtOH, and *n*-Hex. The max antioxidant activity was displayed by *n*-Hex, whereas the extracts of DCM and EtAc displayed intermediate antioxidant activity. EtAc extracts exhibited strong obstruction of α -amylase and α -glucosidase, in comparison with other and control drug (Acarbose). Mostly leaf extracts were good in antibacterial activity with low MIC values in comparison to other extracts and ampicillin. In case of anti-diabetic activity, EtAc extract has significantly reduced glucose levels, but their ZnO NPs were better than extracts. ZnO NPs of EtAc and DCM extracts showed higher anti-inflammatory effects, but their analgesic activities were moderate as compared to Tramadol. According to acute toxicity tests all extracts were safe up to 2000 mg/kg dose. FTIR analysis verifies the existence of multiple functional groups in extracts and NPs. The ZnO NPs of *A. indica* leaf and bark extracts were found better in their pharmacological potential as compared to extracts. The study supports the safe and eco-friendly use of *A. indica* extracts.

Keywords: *Azadirachta indica*, Anti inflammation, ZnO, α -amylase, α -glucosidase, Anti-bacterial, Analgesic.

1. INTRODUCTION

Azadirachta Indica (Neem) has been known in India as a "wonder tree." Neem has been utilized for medicinal purposes in India from the Vedic culture era. Throughout history, this plant and its parts like roots, gums, barks, flowers, fruit and seeds, as well as leaves have been in use as traditional medicine¹. Furthermore, numerous medicinal plants contain herbicidal and anthelmintic properties and are commonly employed to cure intestinal parasites in small ruminants². The role of its phytochemicals is well reported in many physiological conditions, like BP control, management of Parkinson and Alzheimer diseases, preeclampsia, atherosclerosis, and acute renal failure³. Its ZnO based nanoparticles exhibit excellent biocompatibility and demonstrate minimal toxicity, which renders them highly suitable for a wide range of biomedical applications, including applications in pharmaceuticals, cosmetics, and dental restorative materials^{4,5}.

The green synthesis of NPs has various advantages, including simplicity, cost-effectiveness, and reproducibility. These parameters of NPs help them to achieve sustainable development goals in drug development. Extracts derived from coffee, tea, fruits, vegetables, amino acids, and starch can serve as

effective capping and reducing agents for synthesizing stable metal and metal oxide nanoparticles^{6,7}. NPs are also popular in various other industries because of their effective UV filtration properties, super capacitor, high catalytic powers, use in wastewater treatment, and use in gas sensing characteristics⁸. In one study, the leaf extract of *Syzygium cumini* was utilized in making ZnO based NPs. The extract has been acted as the capping agent and produced NPs carrying good anticancer, antioxidant, and antibacterial properties⁹.

According to recent studies, many plant based drugs/extracts have been found effective in treatment of diabetes. Medicinal plants are not only an effective source of hypoglycemic drugs they constitute a rich source of anti-inflammatory constituents, including alkaloids, fatty acids, phenolics, polysaccharides, terpenoids, and other bioactive compounds. The mechanism of each drug in controlling anti-inflammatory response is different from each other and almost all mechanisms have been reported¹⁰.

Diabetes mellitus ranks among the most widespread metabolic disorders, affecting billions of people globally. If remained untreated or unmanaged, it leads to other acute or chronic disorders and creates lot of health complications in body. Currently it is the sixth largest cause of death. Despite advancements in therapy, the results remain unpromising. These therapies have several downsides, including medication resistance and toxicity¹¹. Certain studies have shown that ZnO nanoparticles can enhance insulin activity and regulation production in diabetic patients and improve glucose uptake in muscle cells and reduce stress in muscles¹². Additionally, the extracts showed strong antibiofilm properties, indicating that they may be able to inhibit bacterial strains that form biofilms as well as planktonic ones. The therapeutic potential of Cholistani plants as natural resources for creating novel antibacterial and antibiofilm compounds was observed¹³. The nutritional and therapeutic potential of *C. edulis*, a traditional Cholistani herb, was examined in another study. Phenolics, alkaloids, and other bioactive substances were found abundant in this plant. The extracts of this plant have demonstrated strong antiviral, anti-inflammatory, antipyretic, and anti-diabetic properties with no toxicity even at high doses¹⁴. From another study, variety of extracts were assessed for phytochemical and pharmacological characteristics of *C. polygonoides*. HPLC-PDA and phytochemical screening verified the existence of a variety of beneficial substances, including phenolic.

Significant antibacterial, antibiofilm, antioxidant, antidiabetic (α -glucosidase inhibition), and antiviral properties were displayed by this plant. These findings demonstrate that *C. polygonoides* is a powerful medicinal plant with significant pharmacological value and a wide range of therapeutic possibilities¹⁵. According to another study, antiviral activity of MtOH extracts from eleven Cholistani plants against the Newcastle Disease Virus (NDV) was evaluated and *Achyranthes aspera* extract was found the most efficient (IC₅₀: 3.125 μ g) while *Oxystelma esculentum* was the least effective (IC₅₀: 60 μ g) in controlling the viral growth in embryonated eggs. The study concludes that Cholistani plants are a good source of antiviral substances that protect against NDV¹⁶. Another study supports the idea that leaves and fruits of Cholistani plants represent a rich source of bioactive compounds with antibacterial properties. The best results were obtained from EtOH extract of leaf and aq extract of fruit. The highest antibacterial activity was observed in the leaves of *Datura innoxia* and the fruits of *Physalis minima*. The extracts were often least effective against *K. pneumonia* and most effective against *S. aureus*¹⁷. The present study aims to evaluate the pharmacological potential of *Azadirachta indica* (Neem) leaf and bark extracts for therapeutic applications.

2. METHODOLOGY

2.1. Sample Collection

A. indica leaf and bark was collected from the Cholistan desert Bahawalpur, Pakistan and voucher # (1646) was identified by a taxonomist from the Department of Botany, The Islamia University of Bahawalpur.

2.2. Synthesis of Plant Extract

Freshly collected leaves and bark (20-30 g each) were washed, shade-dried for 3-4 weeks, and finely ground. 1kg of powder was soaked in 2000 mL of analytical grade MtOH for 72 hr in an airtight container. After filtering (Whatman # 1), the residue was re-extracted in 1L MtOH for a further 72hr. The combined filtrates were evaporated to provide crude MtOH extract, which was weighed and kept in Eppendroff tubes at RT. The residue extracted with MtOH was further used in soaking with *n*-Hex, DCM, and EtAc for 72hr before filter with Whatman paper # 1. Filtrates were air-dried, and the crude extracts were weighed, labeled, and stored in Eppendroff tubes at RT.

2.3. Synthesis of ZnO NPs

Zinc sulfate (2.8g) was mixed in 150 mL of distilled water with continuously stirring at 55°C for 5 min. Plant extract (10 mL) from MtOH, EtAc, DCM, or *n*-Hex was added, and the pH was adjusted to 12 using 12 M NaOH. The mixture was heated at 60°C for 5 hrs, cooled to RT, centrifugation was done at 6000 rpm for 10 min at 20°C. The pellet was rinsed with the solvent after the supernatant was removed and dried in oven at 80°C and crushed into ZnO NPs powder¹⁸.

2.4. Characterization

2.4.1. Ultra-violet visible spectroscopy (UV)

The analysis of each plant extract as a reducing agent and the synthesis of ZnO NPs were confirmed by UV-Visible spectrometry. The UV spectrum was recorded after the completion of reaction time in the range of 300-500_{nm} (ZnO NPs exhibit a strong absorption peak) at 10_{nm} resolution¹⁹.

2.4.2. FT-IR spectroscopy

The identification of functional groups in substances is done using the characterization method known as Fourier transform infrared spectroscopy. Air-dried samples NPs were mixed with KBr salt and operated at 4cm⁻¹ in the range of 4000-400 cm⁻¹ ²⁰.

2.5. In Vitro Trials

2.5.1. Antioxidant activity

2.5.1.1. 2, 2-Diphenyl-1-picrylhydrazyl (DPPH) assay

DPPH free radical scavenging technique was employed to evaluate the antioxidant activity leaf extracts. The extract was produced at various concentrations (from a 1 mg/mL stock) using MtOH. An aliquot of 1 ml of 950 μM DPPH solution in MtOH was stirred with the plant extract in MtOH at various concentrations (10, 5, 2.5, 1.25, and 0.625μl). Later on it was incubated at 25°C for 30 min, the mixture was incubated with 1 ml of MtOH in place of the extract, and the absorbance of the test combination was calculated using a spectrophotometry at 517_{nm} against DPPH control. Lower absorbance suggested more antioxidant activity. Ascorbic Acid was used as control²¹.

$$\% \text{ DPPH Scavenging} = [A_{(\text{control})} - A_{(\text{sample})} / A_{(\text{control})}] \times 100$$

The A_(control) stands for blank absorbance and A_(sample) stands for sample absorbance.

2.5.2. α-amylase inhibition studies

Dissolve 1 g of soluble starch in 10 mL of deionized water and gently heat the solution until the starch is fully dissolved. The α-amylase solution (5mg/ml in buffer) was made in phosphate buffer (0.02M, pH

6.9). The DNS reagent is prepared by mixing 438mg of 3, 5-dinitrosalicylic acid with 12g of Na-K tartrate $4\text{H}_2\text{O}$ and 8mL of 2M NaOH in 10 mL of distilled water. Take 200 μL α -amylase solution and mixed with different concentrations (1.25mg, 2.5mg, 5mg, and 10mg/ml) of plant extracts and then add starch solution (200 μL) in each tube. Incubate the mixture for 10 min at 37°C. After the incubation, add 50 μL of DNS reagent to each test tube and boil the mixture for 5min. Take the OD at 540 $_{\text{nm}}$ using an ELISA reader²².

2.5.3. α -glucosidase Inhibition studies

The *p*-nitro phenyl- α D-glucopyranoside (*p*-NPG) substrate used for the inhibition studies and Acarbose was used as the positive control. A 100 mM buffer of Na_3PO_4 with 50 mM NaCl was prepared in dd H_2O with pH 6.9. The variation in absorbance at 405 $_{\text{nm}}$ resulting from the hydrolysis of *p*-NPG was quantified using 96-well plates. An increase in absorbance at 405 $_{\text{nm}}$ indicates the change of *p*-NPG to *p*-nitrophenolate ion. The entire experiment was conducted at 37°C²³.

2.5.4. Antibacterial activity

Four ATCC bacterial strains including *P. aeruginosa*, *E. coli*, *K. pneumonia*, and *S. aureus* were used in the assay. Disc diffusion technique was employed to evaluate antibacterial activity²⁴. On each agar plate 200 μL of fresh bacterial inoculate was spreaded and incubated for 1 hr. After 1hr, presoaked discs were placed and plates were incubated at 37°C for overnight. As positive control, ampicillin was used. Zones of inhibition (ZoI) were measured²⁵. To determine the minimum inhibitory concentration (MIC), 50 μL of nutrient broth and plant extracts were added to the 12th well of a 96-well plate. Following the addition of 100 μL of bacterial culture, the plate was incubated at 37 °C for 24 hours. Subsequently, 50 μL of INT (iodonitrotetrazolium chloride) solution in methanol was added to each well, with a color change indicating bacterial growth²⁶.

2.6. In Vivo Trials

This study used healthy albino female rats measuring 150-250 g. The animals were maintained at the Animal House Facility of the Department of Food Sciences, The Islamia University of Bahawalpur. All animals were kept in conventional laboratory settings 7 days prior to the experiment which included a 12-hr light/dark cycle at 22 ± 1 °C, 35-60 \pm 5% humidity provided free access to feed and water. Prior approval by the Institutional Committee for the Care and Use of Laboratory Animals was taken.

2.6.1. Acute toxicity study

An acute toxicity of *A. indica* leave and Bark extracts was tested on female albino rats weighing 150-250g. The test animals were divided into six groups, each (n=5). Dosages of 200, 500, and 2000mg/kg were given in different groups. Normal saline is given to control groups. Low dosage was administered for 6 hrs to evaluate any toxic effect after high doses were given. All doses were delivered via oral administration. Signs of toxicity and behavioral change such as sweating, convulsions, urination, hyperactivity, alertness, corneal reflex, and mortality in both test and control groups were noted for 24 hrs²⁷.

2.6.2. Anti-diabetic activity

Extracts from *A. Indica* were evaluated for their anti-diabetic properties on Alloxan induced rats. Initially, each animal's weight was considered before injecting Alloxan monohydrate (120 mg/Kg of body weight). Unlimited access to food and feeding bottles containing 5% dextrose solutions were given to animals in order to help them recover from severe hypoglycemia. After 72 hrs, the blood glucose level was monitored by a glucometer. Six groups of diabetic rats (blood sugar levels greater than 150 mg/dL) were treated with respective extracts. There were five animals in each group including +ve and -ve controls.

Blood glucose levels were monitored daily for up to seven days to assess the recovery from hyperglycemia²⁸.

2.6.3. Anti-inflammatory activity

The carrageenan-induced inflammatory test was performed on female albino rats weighing between 150 and 250g. After grouping the rats, the rats' right hind paws were infused with 0.1 ml (1% volume/weight in 0.9% weight/volume NaCl) of carrageenan to elicit acute inflammatory responses. Positive and negative controls were group II and III were, respectively, with the standard dosage of Diclofenac sodium (15 mg/kg) and group I serving as the normal control group (normal saline, 5 ml/kg). Groups that underwent testing were the remaining groups. The sizes of the right hind paws of each rat were measured using a vernier caliper. Diclofenac sodium, a conventional drug, was administered via intraperitoneal injection, while all other doses were delivered orally. Following a 30-minute administration of carrageenan, the extracts were orally administered, and the paw size of each animal was recorded at 1-, 2-, 3-, and 4-hours post-injection. The following formula was then used to calculate the percentage of inhibition²⁹.

$$\% \text{ Inhibition} = \frac{\text{Control mean} - \text{treated mean}}{\text{Control mean}} \times 100$$

2.6.4. Anti-Analgesic activity

2.6.4.1. Tail-immersion test

Weighted between 150 and 200g, young female albino rats were used. They were placed, tails dangling, in individual cylindrical carriers. The bottom portion of the tail, up to 4-5 cm, is marked after the rats are divided into several groups. In addition to an intraperitoneally injection of a conventional medication (tramadol 30 mg/kg), the standard saline (5 ml/kg), EtAc, DCM, MtOH, and *n*-Hex extracts (200,400 mg/kg) of *Azadirachta Indica* leaf and bark were administered orally. Following a 30min injection, the targeted area of the tail was submerged in water freshly heated to a precise temperature of 55 °C. Reaction time was measured using a stopwatch accurate to 0.5 seconds. The flicking of the tail in response to heat was used to indicate the reaction endpoint. To avoid tail damage, a 15-second time limit was applied. The reaction was noted, and the tail was subsequently dried. The tail-flick response was assessed at 30, 60, and 120 minutes. The flicking response was employed to determine the time required for tail withdrawal from the heated water, and the findings were compared to the control group³⁰.

2.7. Statistical Analysis

All data were presented as mean \pm standard error of the mean. Statistical analysis was performed using t-tests and a one-way analysis (ANOVA) of variance was used to examine the data to compare the means among various groups. Values of p less than 0.05 were considered statistically significant. Graphical illustrations were prepared using Microsoft Excel and Graph pad Prism 5 statistical tool (Graph pad software).

3. RESULTS AND DISCUSSION

The current study is aimed at assessing *A. indicia* pharmacological activities both *in vitro* and *in vivo*. In the modern world, a wide range of plants have natural chemicals that have been found and extracted to develop novel treatments.

3.1. Antioxidant activity

Four distinct solvent extract concentrations of *A. Indica* (10mg, 5mg, 2.5mg, and 1.25mg) showed various percentages of inhibition. It's interesting to note that each extract's scavenging activity increased in a concentration-dependent way. The *n*-Hex exhibited the highest level of antioxidant activity (60.19, 85%),

followed by extracts of EtAC, DCM, and MtOH (63.84, 74%, 67.24, 70%, and 62.49, 65%), in that order. At their max concentration of 10mg. Samples with stronger antioxidant activity have lower IC₅₀ values. The extracts of *n-Hex* (0.52) and EtAC (0.63 mg mL⁻¹), DCM (0.67 mg mL⁻¹), and MtOH (0.323 mg mL⁻¹) had the lowest IC₅₀ values, respectively, based on the determined IC₅₀ value. Remarkably, the *n-Hex* extract's IC₅₀ value was likewise less than Ascorbic acid (0.315 ppm). To isolate antioxidant compounds, extraction techniques are crucial. The yield of the extract should be impacted by the polarity of the solvent used during the preparation process. Moreover, the yield may also depend on the solvent of choice and the natural products' solubility. For instance, non-polar solvents like *n-Hex* are appropriate for lipophilic chemicals, which include certain terpenoids and alkaloids. It is common practice to extract some alkaloids, flavonoids, and terpenoid chemicals using EtAC. On the other hand, some flavonols, alkaloids, polyphenols, and saponins are extracted using polar solvents such as MtOH, EtOH, and acetone. DPPH becomes a stable diamagnetic molecule when an electron or hydrogen radical is introduced. A shift in hue from purple to yellow signifies a reduction in the DPPH radical's absorption. This illustrates how free radicals and the antioxidants present in a mixed solution interact³¹. The results revealed that overall, the leave of *A. indica* are good sources of antioxidants and max potential was shown by *n-Hex* extracts, which indicates some lipophilic terpenoids and/or alkaloids are present.

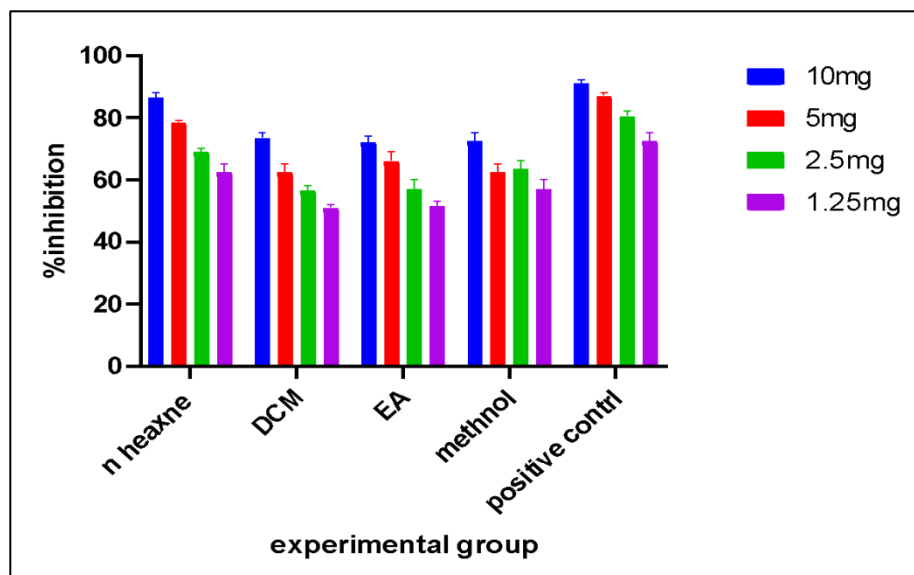


Figure 1. Antioxidant activity of MtOH, DCM, EtAC, and *n-Hex*, extracts of leave of *A. indica* revealed by DPPH assay.

3.2. α -amylase inhibition assay

EtAC extracts from leave and bark have demonstrated strong inhibitory actions against α -amylase, outperforming the common medication Acarbose in higher concentrations. Further, these extracts showed a very low IC₅₀ value, indicating their high potency. IC₅₀ was calculated by graph pad prism. The overall activity trend of leave was EtAC > *n-Hex* > MtOH > DCM (Figure 2a). The overall activity trend of Bark was EtAC > *n-Hex* > DCM > MtOH (Figure 2b). In case of α -amylase inhibition, the leave EtAc extract have shown good inhibition in all concentrations and its IC₅₀ (0.0001018±1) and in case of bark extracts EtAc again had shown best inhibition results with IC₅₀ (0.003295±1).

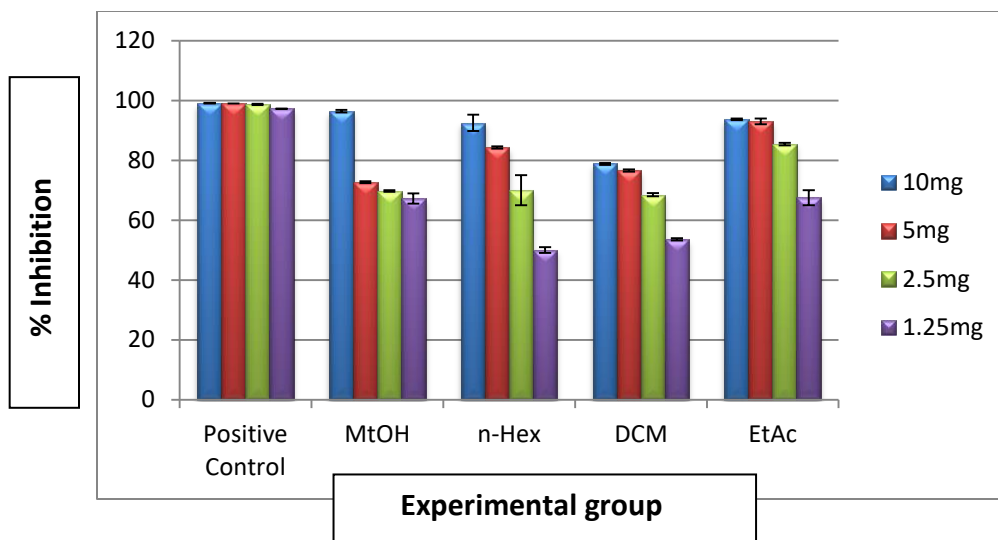


Figure 2(a). α -amylase inhibition effects of leave extracts

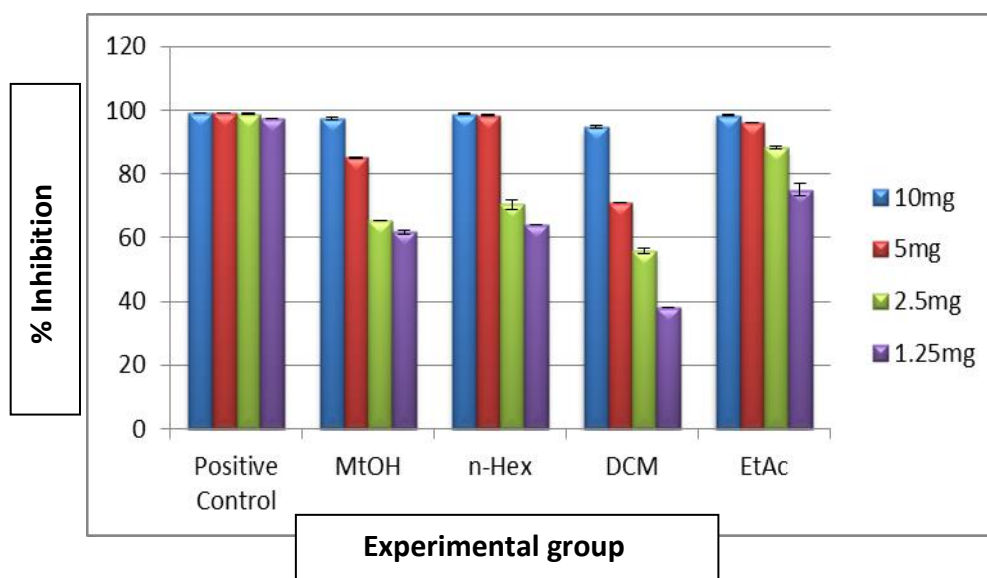


Figure 2(b). α -amylase inhibition effects of bark extracts

3.3. α -glucosidase Inhibition Studies

EtAC extracts from leaf and bark have demonstrated strong inhibitory actions against α -glucosidase, even better than acarbose (known drug) in higher concentrations. Further, these extracts have shown very low IC_{50} value. IC_{50} was calculated by graph pad prism. The overall trend of leaf was EtAC > DCM > MtOH > n-Hex (Figure 3a). The overall trend of bark was EtAC > n-Hex > MtOH > DCM (Figure 3b). In case of α -glucosidase enzyme inhibition studies, the leaf EtAC had shown good inhibition in all tested concentrations and its IC_{50} was (0.0009016 ± 1) . In case of bark extracts again EtAC showed the best results in all test concentrations with IC_{50} (0.02626 ± 1) . In short, leaf extract were more potent than bark.

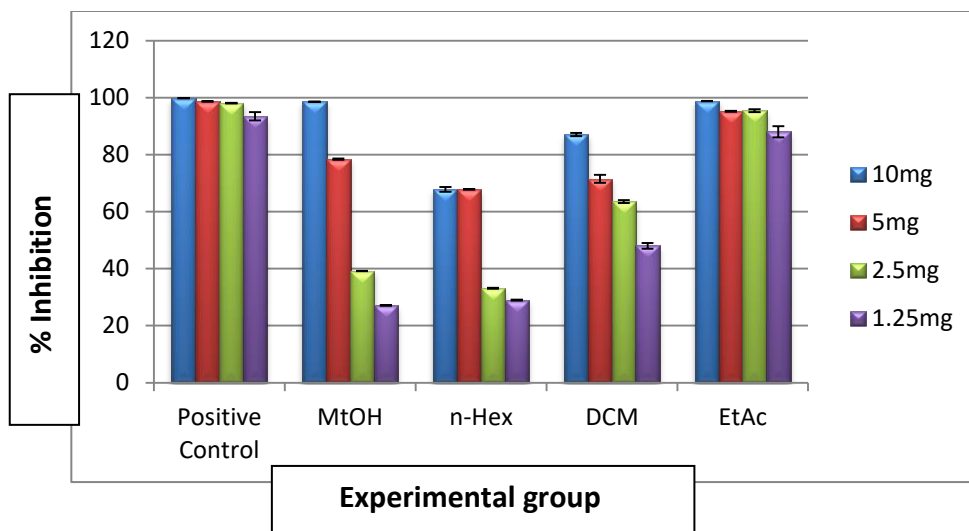


Figure 3(a). α -glucosidase inhibition effects of leaf extracts

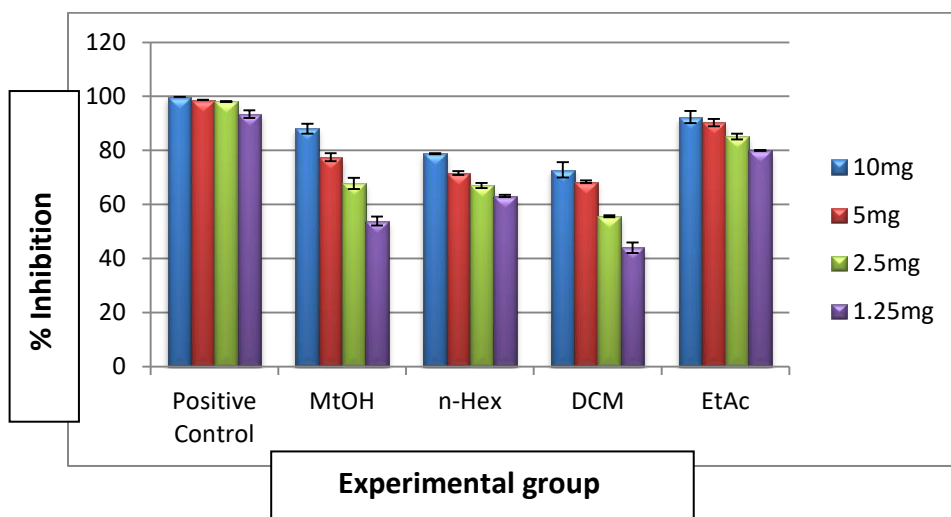


Figure 3(b). α -glucosidase inhibition effects of bark extracts

3.4. Antibacterial activity

In case of *P. aeruginosa* highest activity was shown by DCM with Zol of 8.5mm. EtAC has shown 8.0mm Zol, MtOH expressed Zol of 7.5mm, *n*-Hex showed Zol of 7.0 mm. In case of *K. pneumonia* highest activity was shown by MtOH with Zol of 7.5mm. EtAC Zol 7.0mm, DCM Zol 6mm and *n*-Hex expressed Zol 6mm. In case of *S. aureus* highest activity was shown by EtAC with Zol 9mm. MtOH Zol 9mm, DCM expressed Zol 7.5mm, *n*-Hex expressed Zol 8mm. In case of *E. coli* highest activity was shown by EtAC with Zol 10.5mm. DCM Zol 8mm, MtOH showed Zol 7.0mm and *n*-Hex expressed Zol 7mm. According to these results, *A. indica* leaf extracts have shown higher antibacterial potential. In leaf DCM extract has shown highest activity against *P. aeruginosa* with Zol 8.5mm. In case of *K. pneumonia* highest activity was shown by MtOH with Zol 7.5mm. In case of *S. aureus* and *E. coli* highest activity was shown by EtAC with Zol of 9mm and 10.5mm. The MIC of each positive extract was calculated and compared with ampicillin. ZnO antibacterial capabilities are based on several parameters,

including particle size, concentration, shape, and surface area of salt. Studies have shown that smaller ZnO particles with a greater specific surface area suppress bacterial growth more efficiently³².

Table 1. Antibacterial potential and MIC of different extract of *A. indica* leave

Extract of leave	<i>P. aeruginosa</i>		<i>K. pneumonia</i>		<i>Staph aureus</i>		<i>E. coli</i>	
	Zol±SEM	MIC	Zol±SEM	MIC	Zol±SEM	MIC	Zol±SEM	MIC
MtOH	7.50±0.5	--	7.50±0.50	2500	9.00±0.00	2500	7.00±1.00	--
DCM	8.50±0.50	2500	6.00±0.00	--	7.50±0.50	--	8.00±0.00	--
<i>n</i> -Hex	7.00±1.00	--	6.00±0.00	--	8.00±1.00	--	7.00±0.00	--
EtAc	8.00±1.00	--	7.00±1.00	625	9.00±1.00	2500	10.5±0.50	1250
Control	15.0±1.00		14.5±0.50		15.0±1.00		14.5±0.50	

3.5. Acute toxicity study

A. indica crude extracts were given to several groups of female albino rats at doses of 200, 500, 1000, and 2000 mg/kg BW. These doses were selected in accordance with Organization for Economic Co-operation and Development (OECD) rules and the efficacy of prior studies. The animals were attentively observed during the following 14 days following the administration of dosages. All behavioral abnormalities, including convulsions, corneal reflex, perspiration, allergic response, hyperactivity, and mortality, were monitored in the animals. Until the dose level of 2000 mg/kg, no significant morbidity or death was noted in the acute toxicity trial. Itching in the bark extract and moderate perspiration in the Leaf extract were noted at dosage levels of 2000 mg/kg. Thus, it can be said that using *A. indica* up to 2000 mg/kg is safe. Moreover, it is noted that the LD₅₀ of *A. indica* is greater than 2000mg/kg.

3.6. In Vivo anti-diabetic activity of extract

The EtAc extract showed highly significant anti-diabetic activity of leave and barks compared to MtOH, DCM and *n*-Hex extracts. On the first, third, and fifth days, there were no significant differences in controlling blood glucose from MtOH, DCM, and *n*-Hex extract and the +ve control group. The overall trend for anti-diabetic activity of leaf extract is *n*-Hex > DCM > EtAc > MtOH (Figure 4a). The overall trend for anti-diabetic activity of bark extract is DCM > *n*-Hex > EtAc > MtOH (Figure 4b). According to these results nonpolar and partially polar extracts were more effective in controlling diabetes in rat models. These results are slightly different than enzyme inhibition studies.

3.7. Anti-diabetic Activity of NPs

The EtAc NPs showed highly significant antidiabetic activity of leave and barks compared to MtOH and *n*-Hex extracts. On the first, third, and fifth days, there were no significant differences between MtOH, DCM, and *n*-Hex extract and the +ve control group. However, there was a minor improvement on day seven. The overall trend for anti-diabetic activity of leave NPs is DCM > *n*-Hex > EtAc > MtOH (Figure 5a). The overall trend for anti-diabetic activity of bark NPs is EtAc > DCM > *n*-Hex > MtOH (Figure 5b). According to *In Vivo* studies, NPs are more effective as compared to extracts; further, partially polar solvents have produced more effective anti-diabetic agent(s).

3.8. Anti-Inflammatory

The MtOH leaf extract of *A. indica* showed remarkable anti-inflammatory properties. The 200 and 400 mg/kg dosages are both quite successful. *A. indica* leaf extract EtAc demonstrates strong anti-inflammatory properties. All doses show a significant reduction in paw size, especially at the dose of 400mg/kg; Paw size reduced significantly. The overall trend of *A. indica* leave extracts against inflammation is MtOH> EtAc> DCM >n-Hex (Figure 6a). In case of leave NPs the trend was MtOH>EtAc>n-Hex >DCM (Figure 6b). The overall anti inflammation trend of bark extract was DCM>MtOH>EtAc >n-Hex (Figure 6c). The overall trend of *A. indica* bark extract against inflammation is EtAc>DCM>n-Hex >MtOH (Figure 6d). Medicinal plants include secondary metabolites such phenols, flavonoids, alkaloids, and saponins, which contribute to their anti-inflammatory properties. They can trigger several pro-inflammatory mediators, including mast cells, macrophages, lymphocytes, and neutrophils. secary metabolites may be responsible for suppressing pro-inflammatory enzymes (COX and PLA2)³³. The anti-inflammatory potential has been investigated using several extracts of *A. indica* leave, bark and their NPs. The overall trend of leave extracts against inflammation was MtOH> EtAc> DCM >n-Hex but the trend was slightly different in case of leave NPs i.e. MtOH>EtAc>n-Hex>DCM. The overall anti inflammation trend of bark extract was DCM>MtOH>EtAc >n-Hex. The overall trend of *A. indica* bark NPs against inflammation was EtAc>DCM>n-Hex >MtOH. In anti-inflammation studies, it has been seen that polar and partially polar extracts are more effective. Similarly, NPs are more effective than extracts. The results of this study indicate that polar and partially solvents are the best to yield anti-inflammatory agent(s).

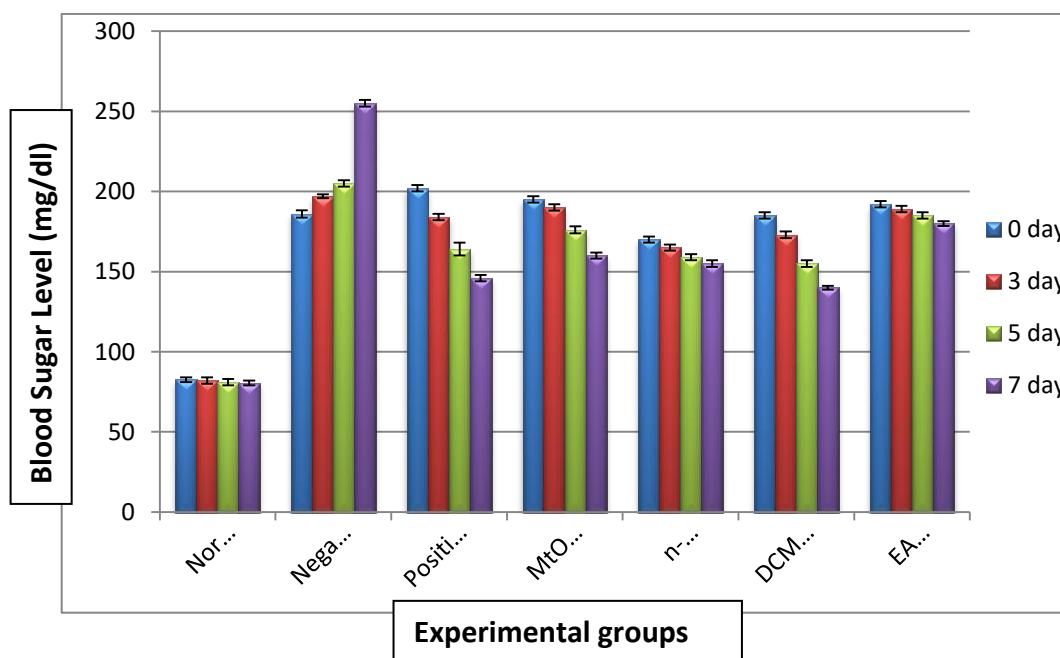


Figure 4(a). Anti-diabetic activity of *A.indica* leaves extract

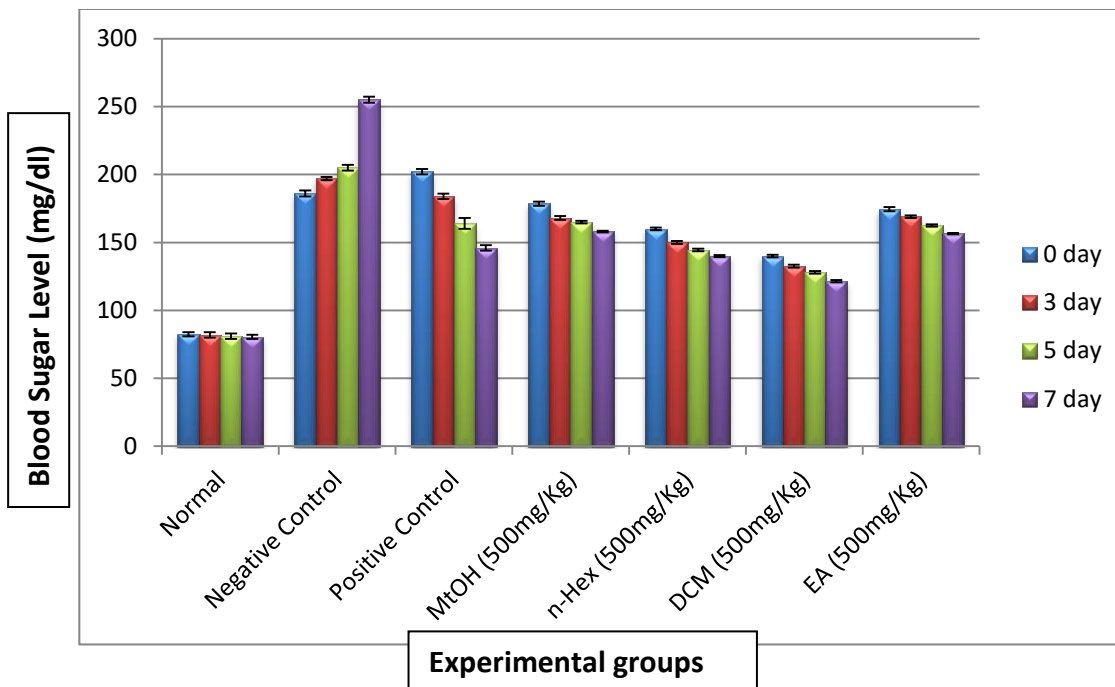


Figure 4(b). Anti-diabetic activity of *A. indica* bark extract

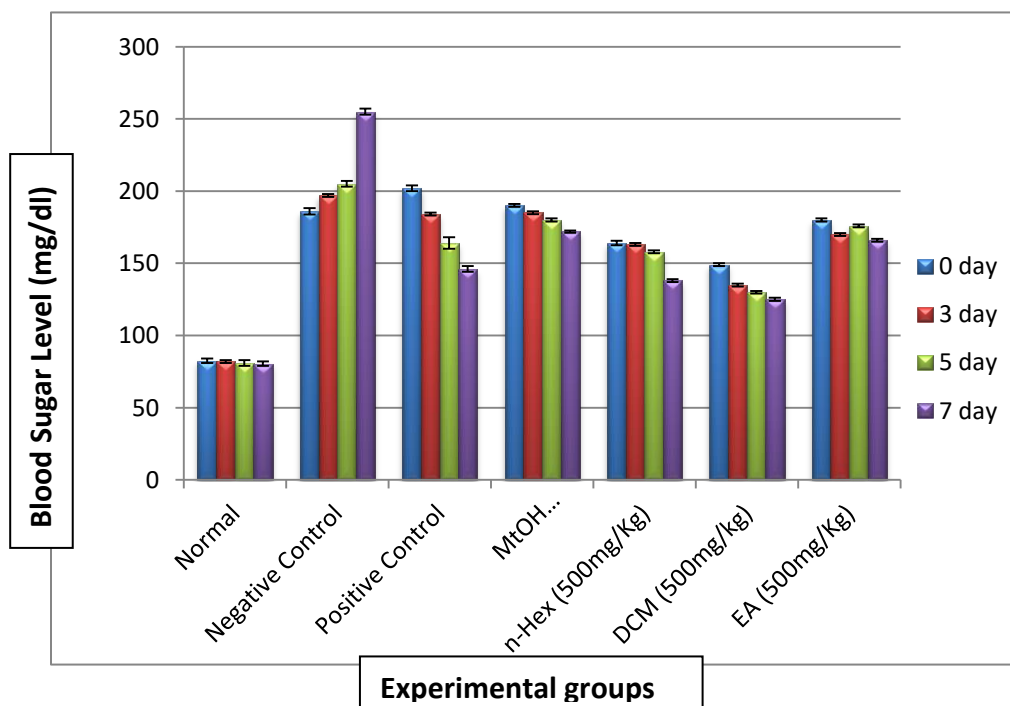


Figure 5(a). Anti-diabetic activities of *A. indica* leave NPs

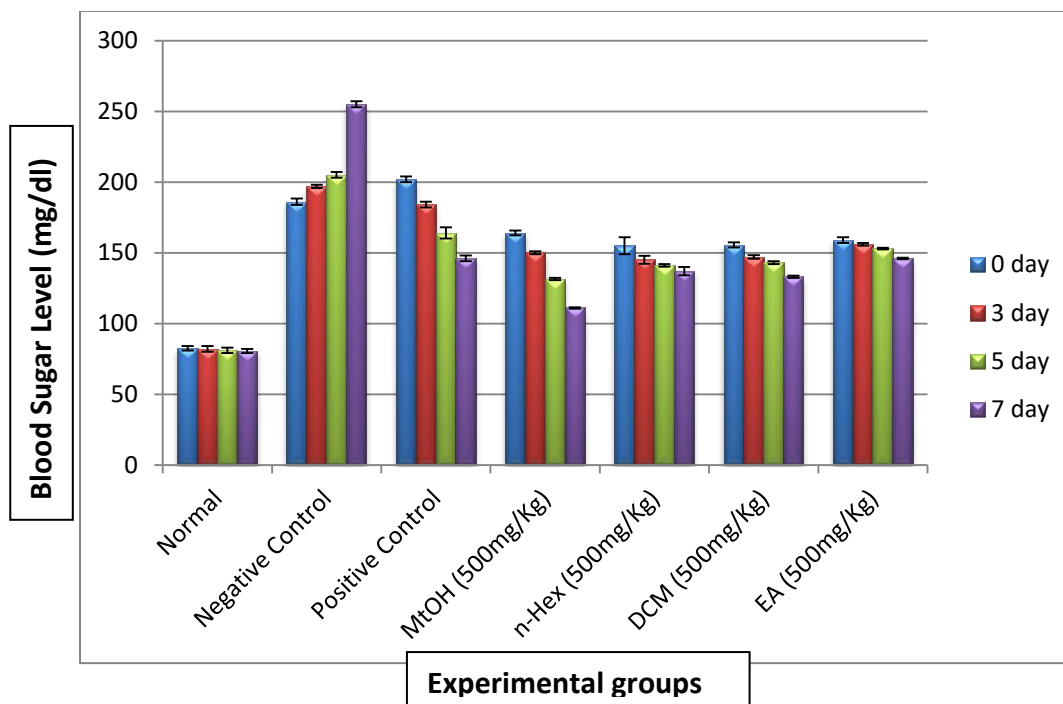


Figure 5(b). Anti-diabetic activity of *A. indica* bark NPs

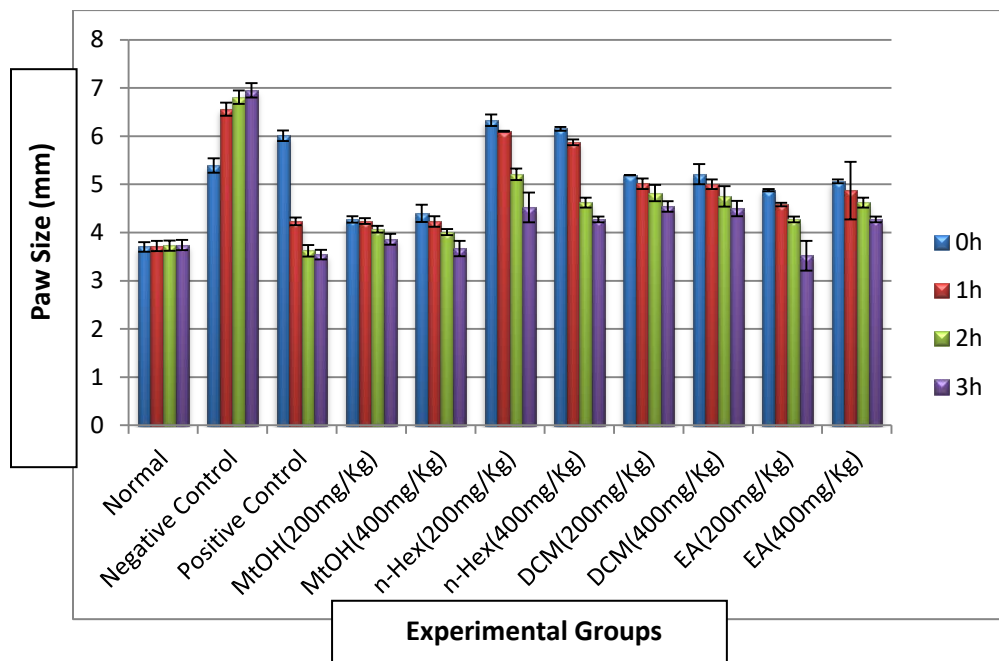


Figure 6(a). Anti-inflammatory effects of leaf extract of *A. indica*

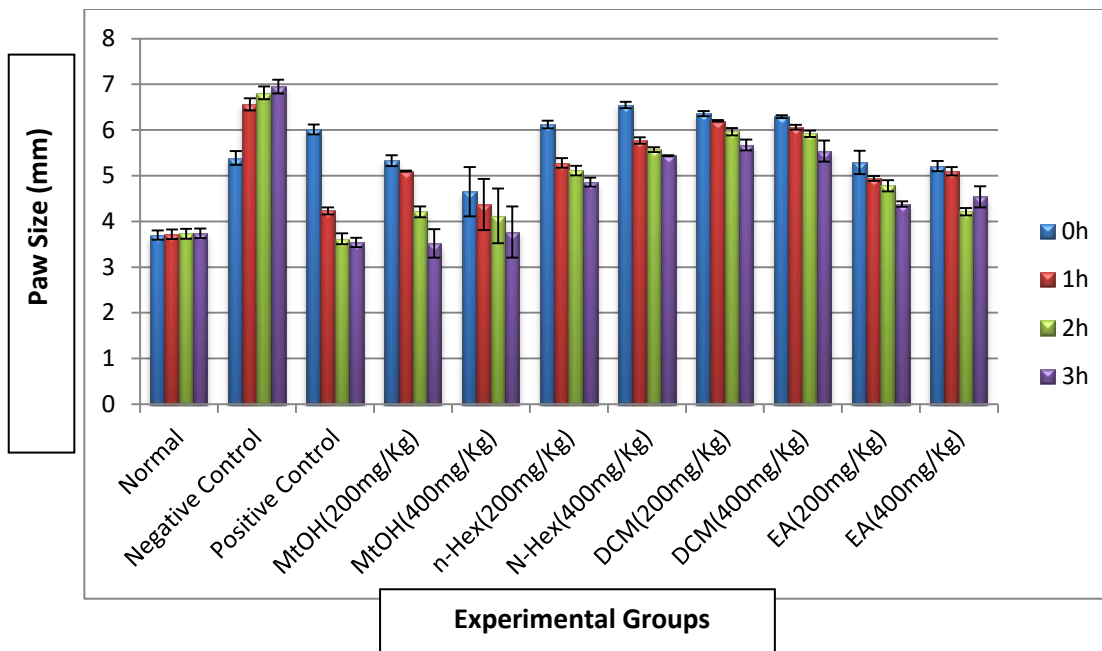


Figure 6(b). Anti-inflammatory effects of leaf NPs of *A. indica*

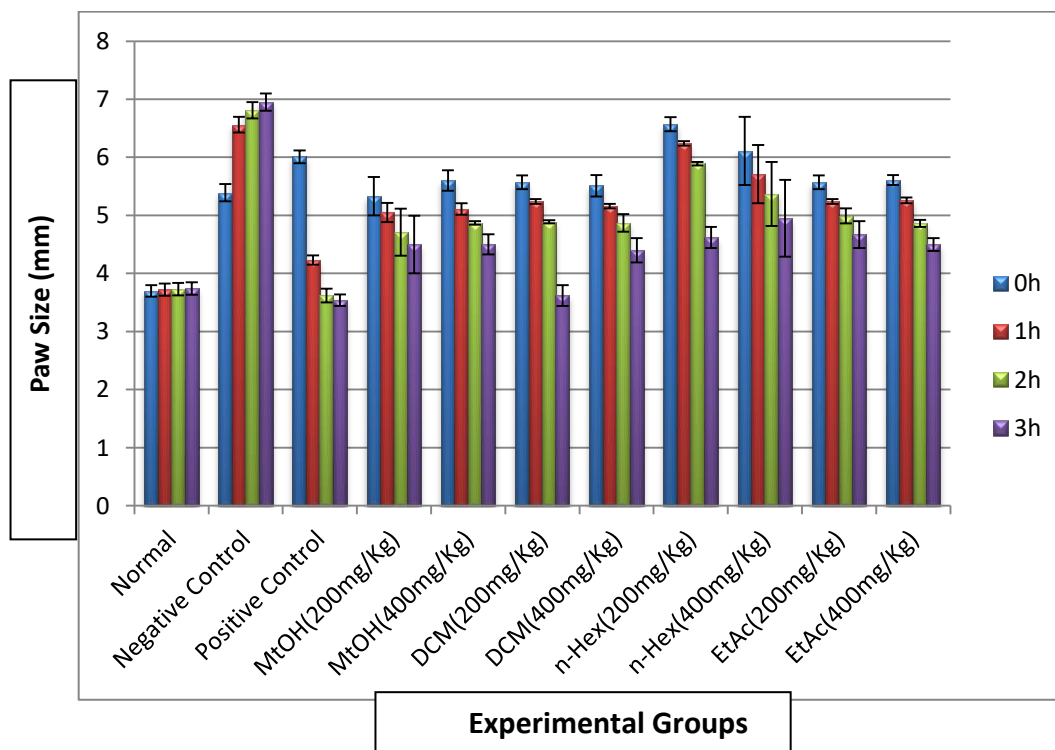


Figure 6(c). Anti-inflammatory effects of bark extract of *A. indica*

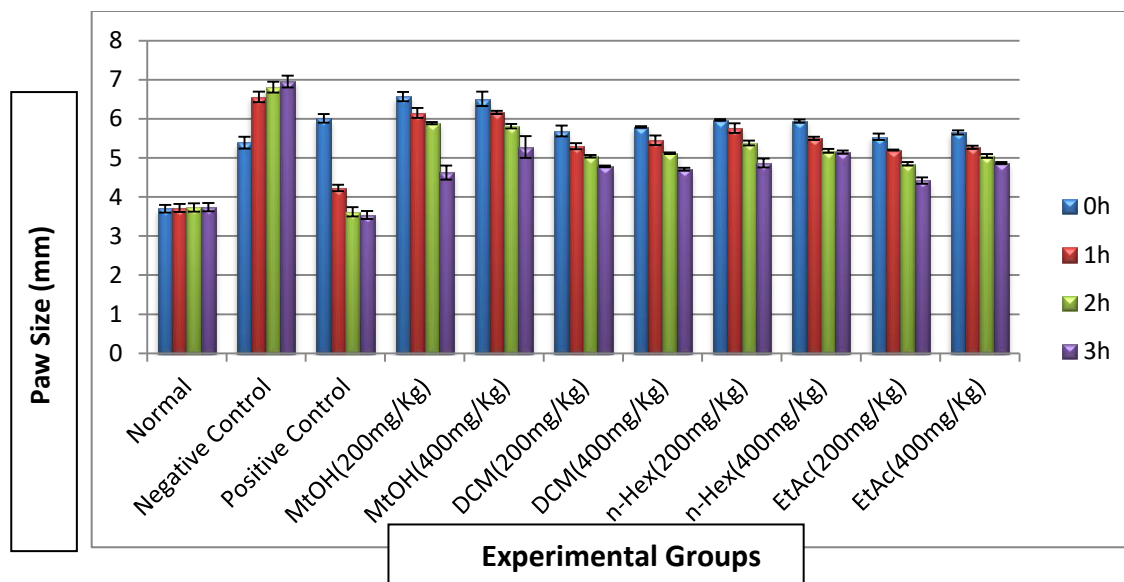


Figure 6(d). Anti-inflammatory effects of bark NPs of *A. indica*

3.9. Anti-Analgesic Activity

MtOH at a 200mg/kg dose level showed a significant increase in animal response time after oral administration. The reaction times (sec, \pm SEM) of different treatment groups measured before and after dosing at various points: 0, 30, 60, 90, and 120 sec. The overall trend of *A. indica* leave extract against analgesic is EtAc>MtOH>DCM>n-Hex (Figure 7a). The overall trend of *A. indica* bark extract against analgesic is EtAc>MtOH>n-Hex >DCM (Figure 7b). This study exhibited significant pain-relieving potential of *A. indica* extracts and NPs. The NPs were slightly better than extract. This study uniquely evaluates neem leaf and bark extracts together with their green-synthesized Zn NPs in an integrated design. It links variations in phytochemical content to differences in NPs formation and biological activity. The side-by-side comparison reveals how each plant part shapes NPs properties and *in vivo* effects. These findings provide new insight for optimizing *A. indica* based green nanotherapeutics.

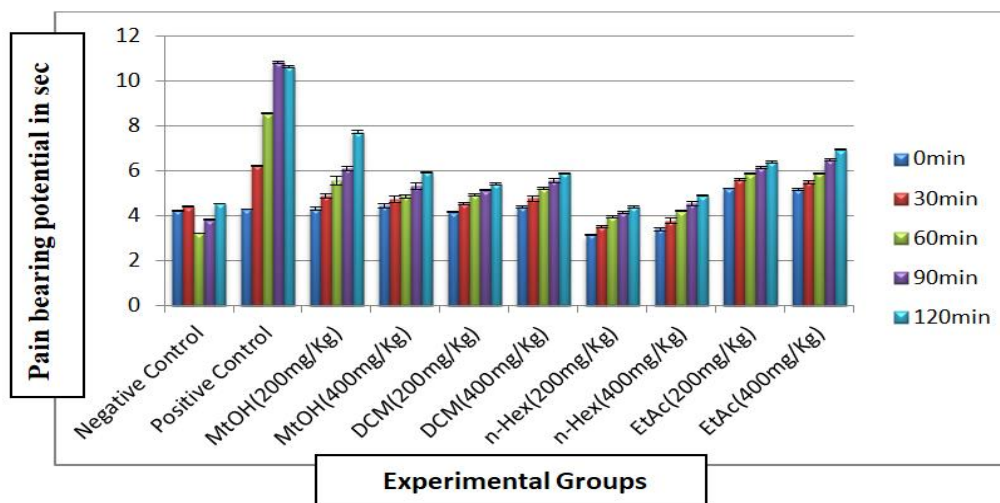


Figure 7(a). Analgesic effects of leave extract of *A. indica*

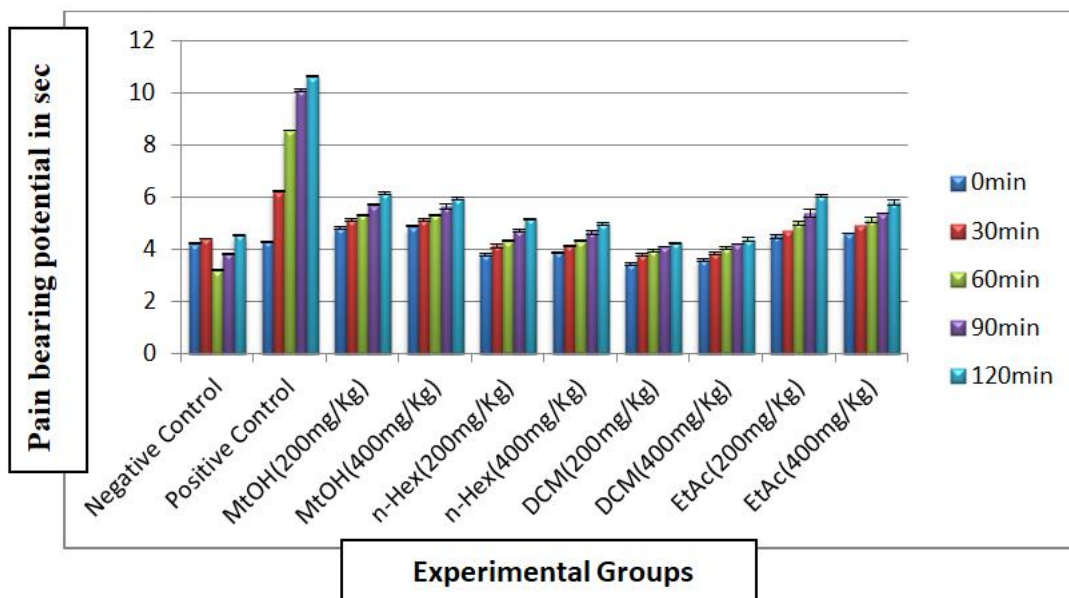


Figure 7(b). Analgesic effects of bark extract of *A. indica*

5. CONCLUSION

A. indica contains anti-inflammatory, antidiabetic, and antioxidant agents, making it effective natural remedy. The most effective extracts were EtAc, MtOH, and DCM. The max antioxidant activity was displayed by *n*-Hex, whereas the EtAc extracts exhibited strong inhibitory potentials of α -amylase and α -glucosidase. Mostly leaf extracts were good in antibacterial activity with min MIC values. In case of anti-diabetic activity, EtAc extract was good, but their ZnO NPs were much better than extracts. ZnO NPs of EtAc and DCM extracts showed higher anti-inflammatory effects, but their analgesic activities were moderate as compared to Tramadol. According to acute toxicity tests all extracts were safe up to 2000 mg/kg dose. The ZnO NPs of *A. indica* leave and bark extracts were found better in their pharmacological potential as compared to extracts. The study supports the safe and eco-friendly use of *A. indica* extracts.

REFERENCES

- [1] Islas, J. F.; Acosta, E.; G-Buentello, Z.; Delgado-Gallegos, J. L.; Moreno Treviño, M. G.; Escalante, B.; et al. An Overview of Neem (*Azadirachta indica*) and Its Potential Impact on Health. *J. Funct. Foods* 2020, 74, 104171. <https://doi.org/10.1016/j.jff.2020.104171>
- [2] Degla, L. H.; Kuseu, J.; Olounlade, P. A.; Attindehou, S.; Hounzangbe-Adote, M. S.; et al. Use of Medicinal Plants as Alternative for the Control of Intestinal Parasitosis: Assessment and Perspectives. *Agrobiol. Rec.* 2022, 7 (1), 1–9. <https://doi.org/10.47278/journal.abr/2021.011>
- [3] Munteanu, I. G.; Apetrei, C. Analytical Methods Used in Determining Antioxidant Activity: A Review. *Int. J. Mol. Sci.* 2021, 22 (7), 3380. <https://doi.org/10.3390/ijms22073380>

- [4] Rani, N.; Yadav, S.; Mushtaq, A.; Rani, S.; Saini, M.; Rawat, S.; Gupta, K.; Saini, K.; Maity, D. Azadirachta indica Peel Extract-Mediated Synthesis of ZnO NPs for Antimicrobial, Supercapacitor, and Photocatalytic Applications. *Chem. Pap.* 2024, 78 (6), 3687–3704. <https://doi.org/10.1007/s11696-024-03340-6>.
- [5] Mohammed, Y. H. I.; Alghamdi, S.; Jabbar, B.; Marghani, D.; Beigh, S.; Abouzied, A. S.; Khalifa, N. E.; Khojali, W. M. A.; Huwaimel, B.; Alkhalifah, D. H. M.; Hozzein, W. N. Green Synthesis of Zinc Oxide NPs Using Cymbopogon citratus Extract and Its Antibacterial Activity. *ACS Omega* 2023, 8 (35), 32027–32042. <https://doi.org/10.1021/acsomega.3c03908>
- [6] Hemlata; Meena, P. R.; Singh, A. P.; Tejavath, K. K. Biosynthesis of Silver NPs Using Cucumis prophetarum Aqueous Leaf Extract and Their Antibacterial and Antiproliferative Activity against Cancer Cell Lines. *ACS Omega* 2020, 5 (10), 5520–5528. <https://doi.org/10.1021/acsomega.0c00155>.
- [7] Tsegaye, G.; Kiflie, Z.; Mekonnen, T. H.; Jida, M. Synthesis and Characterization of Coffee Husk Extract (CHE)-Capped ZnO NPs and Their Antimicrobial Activity. *Biomass Convers. Bioref.* 2023. <https://doi.org/10.1007/s13399-023-04908-0>
- [8] Srikhao, N.; Ounkaew, A.; Kasemsiri, P.; Theerakulpisut, S.; Okhawilai, M.; Hiziroglu, S. Green Synthesis of Silver NPs Using the Extract of Spent Coffee Used for Paper-Based Hydrogen Peroxide Sensing Device. *Sci. Rep.* 2022, 12 (1), 20099. <https://doi.org/10.1038/s41598-022-22067-6>
- [9] Yadav, A.; Kumar, H.; Kumar, P.; Rani, G.; Maken, S. Syzygium cumini Leaf Extract Mediated Green Synthesis of ZnO NPs: A Sustained Release for Anticancer, Antimicrobial, Antioxidant, and Anti-Corrosive Applications. *J. Mol. Struct.* 2025, 141017. <https://doi.org/10.1016/j.molstruc.2024.141017>.
- [10] Mubashir, A.; Ghani, A.; Mubashar, A. Common Medicinal Plants Effective in Peptic Ulcer Treatment: A Nutritional Review. *Int. J. Agric. Biosci.* 2022, 11 (2), 70–74. <https://doi.org/10.47278/journal.ijab/2022.010>
- [11] Ashraf, M.; Ahmad, N.; Akbar, F.; Fazal, H.; Ali, L.; et al. Time and Concentration-Dependent Differential Antioxidant Potential in the Gum of Medicinally Important *Araucaria heterophylla*. *Agrobiol. Rec.* 2023, 13 (1), 44–52. <https://doi.org/10.47278/journal.abr/2023.024>
- [12] Suresh, P.; Doss, A.; Praveen Pole, R.; Devika, M. Green Synthesis, Characterization and Antioxidant Activity of Bimetallic (Ag-ZnO) NPs Using *Capparis zeylanica* Leaf Extract. *Biomass Convers. Bioref.* 2024, 14 (14), 16451–16459. <https://doi.org/10.1007/s13399-023-03743-7>
- [13] Shahzad, M. I.; Anwar, S.; Aslam, J.; Manzoor, A.; Ashraf, H.; Saba, N.; Arshad, M. Antibacterial and Antibiofilm Activities from Extracts of Selected Cholistani Plants. *Front. Chem. Sci.* 2022, 3 (2), 32–45. <https://doi.org/10.52700/fcs.v3i1.50>
- [14] Gillani, B.; Tariq, S.; Shahzad, M. I.; Fatima, T.; Locatelli, M.; Cai, X.; Ahmad, A. Phytochemical Composition and Therapeutic Potential of *Caralluma edulis*, a Cholistani Plant. *J. King Saud Univ. Sci.* 2024, 36 (11), 103519. <https://doi.org/10.1016/j.jksus.2024.103519>
- [15] Sadiq, F.; Shahzad, M. I.; Tariq, S.; Locatelli, M.; Tartaglia, A.; Kalyar, G. M.; Saeed, I. Ethnopharmacological and Phytochemical Evaluations of Desert Plant *Calligonum*

- polygonoides*–Polygonaceae. *Pak. J. Bot.* 2025, 57 (1), 249–262. [http://dx.doi.org/10.30848/PJB2025-1\(25\)](http://dx.doi.org/10.30848/PJB2025-1(25))
- [16] Shahzad, M. I.; Ashraf, H.; Arshad, M.; Parveen, S.; Aslam, A.; Naz, N.; Mukhtar, M. Study of Antiviral Potential of Cholistani Plants against Newcastle Disease Virus. *Pak. J. Zool.* 2019, 51 (1), 1–4. <http://dx.doi.org/10.17582/journal.pjz/2019.51.1.SC11>
- [17] Hussain, T.; Fatima, I.; Rafay, M.; Shahzad, M. I.; Abdullah, M.; Bano, S.; Ruby, T. Comparison of Antibacterial Potential from Leaves and Fruits of Different Herbs and Shrubs of Family Solanaceae. *Int. J. Agric. Biol.* 2015, 17 (6), 1249–1254. <http://dx.doi.org/10.17957/IJAB/15.0039>
- [18] Sadiq, H.; Sher, F.; Sehar, S.; Lima, E. C.; Zhang, S.; Iqbal, H. M.; Zafar, F.; Nuhanović, M. Green Synthesis of ZnO NPs from *Syzygium cumini* Leaf Extract with Robust Photocatalysis Applications. *J. Mol. Liq.* 2021, 335, 116567. <https://doi.org/10.1016/j.molliq.2021.116567>
- [19] Abel, S.; Tesfaye, J. L.; Shanmugam, R.; Dwarampudi, L. P.; Lamessa, G.; Nagaprasad, N.; Benti, M.; Krishnaraj, R. Green Synthesis and Characterizations of Zinc Oxide (ZnO) NPs Using Aqueous Leaf Extracts of Coffee (*Coffea arabica*) and Its Application in Environmental Toxicity Reduction. *J. Nanomater.* 2021, 2021, 3413350. <https://doi.org/10.1155/2021/3413350>
- [20] Enders, A. A.; North, N. M.; Fensore, C. M.; Velez-Alvarez, J.; Allen, H. C. Functional Group Identification for FTIR Spectra Using Image-Based Machine Learning Models. *Anal. Chem.* 2021, 93 (28), 9711–9718. <https://doi.org/10.1021/acs.analchem.1c00867>
- [21] Brand-Williams, W.; Cuvelier, M. E.; Berset, C. L. W. T. Use of a Free Radical Method to Evaluate Antioxidant Activity. *LWT-Food Sci. Technol.* 1995, 28 (1), 25–30. [https://doi.org/10.1016/S0023-6438\(95\)80008-5](https://doi.org/10.1016/S0023-6438(95)80008-5)
- [22] Ahmed, M. U.; Ibrahim, A.; Dahiru, N. J.; Mohammed, H. S. Alpha Amylase Inhibitory Potential and Mode of Inhibition of Oils from *Allium sativum* (Garlic) and *Allium cepa* (Onion). *Clin. Med. Insights Endocrinol. Diabetes* 2020, 13, 1–8. <https://doi.org/10.1177/1179551420963106>
- [23] Feng, Y.; Nan, H.; Zhou, H.; Xi, P.; Li, B. Mechanism of Inhibition of α -Glucosidase Activity by Bavachalcone. *Food Sci. Technol.* 2022, 42, e123421. <https://doi.org/10.1590/fst.123421>
- [24] Demissie, M. G.; Sabir, F. K.; Edossa, G. D.; Gonfa, B. A. Synthesis of Zinc Oxide NPs Using Leaf Extract of *Lippia adoensis* (Koseret) and Evaluation of Its Antibacterial Activity. *J. Chem.* 2020, 2020, 7459042. <https://doi.org/10.1155/2020/7459042>
- [25] Liu, G.; Zhou, Y.; Xu, Z.; Bao, Z.; Zheng, L.; Wu, J. Janus Hydrogel with Dual Antibacterial and Angiogenesis Functions for Enhanced Diabetic Wound Healing. *Chin. Chem. Lett.* 2023, 34 (4), 107705. <https://doi.org/10.1016/j.ccl.2022.07.048>
- [26] Arshad, M.; Ruby, T.; Shahzad, M. I.; Alvi, Q.; Aziz, M. Antimicrobial Activity of Oil Extracted from *Saarahardwickii*. *Braz. J. Biol.* 2022, 84 (1), 1–7. <https://doi.org/10.1590/1519-6984.253508>
- [27] Kharchoufa, L.; Bouhrim, M.; Bencheikh, N.; El Assri, S.; Amirou, A.; Yamani, A.; Choukri, M.; Mekhfi, H.; Elachri, M. Acute and Subacute Toxicity Studies of the Aqueous Extract from *Haloxylon scoparium* Pomel (*Hammada scoparia* (Pomel)) by Oral Administration in Rodents. *Biomed Res. Int.* 2020, 2020, 4020647. <https://doi.org/10.1155/2020/4020647>

- [28] Elbermawi, A.; Ali, A. R.; Amen, Y.; Ashr, A.; Ahmad, K. F.; Mansour, E.-S. S.; Halim, A. F. Anti-Diabetic Activities of Phenolic Compounds of *Alternaria* sp., an Endophyte Isolated from the Leaves of Desert Plants Growing in Egypt. *RSC Adv.* 2022, 12 (38), <https://doi.org/10.1039/D2RA02532A>
- [29] Jisha, N.; Vysakh, A.; Vijeesh, V.; Latha, M. S. Anti-Inflammatory Efficacy of MtOHic Extract of *Muntingia calabura* L. Leaves in Carrageenan-Induced Paw Edema Model. *Pathophysiology* 2019, 26 (1), 323–330. <https://doi.org/10.1016/j.pathophys.2019.08.002>
- [30] Obese, E.; Biney, R. P.; Henneh, I. T.; Adakudugu, E. A.; Anokwah, D.; Agyemang, L. S.; Ameyaw, E. O. The Anticonvulsant Effect of Hydroethanolic Leaf Extract of *Calotropis procera* (Ait) R. Br. (Apocynaceae). *J. Chem.* 2021, 2021, 5566890. <https://doi.org/10.1155/2021/5566890>
- [31] Nwozo, O. S.; Effiong, E. M.; Aja, P. M.; Awuchi, C. G. Antioxidant, Phytochemical, and Therapeutic Properties of Medicinal Plants: A Review. *Int. J. Food Prop.* 2023, 26 (1), 359–388. <https://doi.org/10.1080/10942912.2022.2157425>
- [32] Mendes, C. R.; Dilarri, G.; Forsan, C. F.; Sapata, V.; de M. R.; Lopes, P. R. M.; de Moraes, P. B.; Montagnolli, R. N.; Ferreira, H.; Bidoia, E. D. Antibacterial Action and Target Mechanisms of Zinc Oxide NPs against Bacterial Pathogens. *Sci. Rep.* 2022, 12 (1), 2658. <https://doi.org/10.1038/s41598-022-06657-y>
- [33] Wang, Q.; Kuang, H.; Su, Y.; Sun, Y.; Feng, J.; Guo, R.; Chan, K. Naturally Derived Anti-Inflammatory Compounds from Chinese Medicinal Plants. *J. Ethnopharmacol.* 2013, 146 (1), 9–39. <https://doi.org/10.1016/j.jep.2012.12.013>