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# Pharmacognostic and Pharmacological Study of *"Azadirachta Indica"* Bark Extracts

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**Abstract:** *Azadiracta indica A. Juss (Meliaceae) commonly known as Neem,is found throughout India and is known to have many wonderous properties from ancient times.These A.indica shows different medicinal properties like antiulcerogenic, hypoglycemic, insecticidal, spermicidal actions. The stem bark was studied for morphological as well as microscopical characteristics. The present work was carried out on bark of Azadirachta indica belonging to family Meliaceae. The study was done on pharmacognostic, phytochemical and pharmacological studies on stems bark of Azadirachta indica collected from Sangamner of Ahmednagar district. Preliminary phytochemical screening of extract was done.Some part of extract was then used to study pharmacological screening of anti-anxiety activity.From the study of phytochemical and pharmacological investigation of Azadirachta indica bark it was found that different chemicals are present as glycosides, alkaloids, tannins, fats, steroids, proteins, etc. Azadirachta indica stem bark supports the elimination of related anxiety associated materials. Therefore, drawing conclusions from this research suggests that Azadirachta indica hydroethanolice etract were found to be with good antianxiety like activity in elevated plus maze model. Furthermore, this current study confirms the conventional utilisation of Azadirachta indica bark as a pain reliever by showing that its hydroethanolic determine has noteworthy analgesic properties for various dose amounts, with the greatest analgesic movement observed at a 60-minute time interval. More research is required, nevertheless, to determine how precisely it exerts its analgesic effects.*

**Keywords:** *Azadiracta indica,* anti-anxiety, hydroethanolic, analgesic effects

## I. INTRODUCTION

Ayurveda, the ancient Indian medicine system, indeed stands out as one of the primary alternative and complementary medicine technologies globally. It has garnered attention for its holistic approach to health and well-being, focusing on the balance between mind, body, and spirit.One of the distinctive features of Ayurveda is its reliance on natural remedies, particularly herbs. India, with its diverse climate and terrain, boasts a rich repository of medicinal plants, earning it the moniker "Emporium of Medicinal Plants." These plants serve as the foundation for a significant portion of Ayurvedic medicines.In the context of prevalent health issues like inflammation and anxiety disorders, Ayurveda offers various herbal remedies that aim to address these concerns without causing severe adverse effects commonly associated with prolonged use of conventional medicines.For instance, herbs like turmeric (Curcuma longa) and ginger (Zingiber officinale) has strong anti-inflammatory characteristics and are frequently included into Ayurvedic remedies to alleviate inflammation and associated symptoms. Similarly, adaptogenic herbs like ashwagandha (Withania somnifera) and brahmi (Bacopa monnieri) are renowned for their ability to combat stress and anxiety by promoting relaxation and supporting the nervous system. While Ayurvedic remedies can If you want to successfully manage these health conditions, you must emphasize the importance of consulting qualified practitioners for personalized treatment plans. Additionally, integrating Ayurveda with conventional medical approaches under supervision can offer a comprehensive approach to health and well-being, ensuring optimal outcomes while minimizing risks.


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## II. METHODOLOGY-CHAPTER-WISE DETAILS OF PROPOSED RESEARCH:

**Phase 1:** Literature review

The chosen heterocyclic scaffolds were the subject of an extensive literature analysis.

**Phase 2:** Plant Material

**Phase 3**: Pharmacognostic Study, Evaluation of Physical Constant

**Phase 4**: Phytochemical studies

**Phase 4**: Pharmacological Studies- to examine the anxiolytic and analgesic activity of the isolated phytoconstituents of *Azadirachta indica* bark.

**SOURCE OF DATA:** Data will be obtained from the experimental work, which includes laboratory based animals studies and evaluation of various parameters.

## III. METHODS: ANXIOLYTIC ACTIVITY-ELEVATED PLUS MAZE TEST:

**ANIMALS:Table no.1-Animals**

| | |
|---|---|
| Species | Rat |
| No of animals in each group | N=06 |
| No of groups | Three. |
| Vehicle of herbal drugs | Distilled water |
| Vehicle of standard drugs | Distilled water |

**DRUGS AND TREATMENT:**

Group I: vehicle control, Group II: diazepam, Group III: test drug dose I

**ANALGESIC ACTIVITY-**Experimental Design

We utilised healthy male Wister albino rats that weighed 180-220 g. Each of the six groups, consisting of six albino rats each, was selected at random: -

Group I-gave the patient a mouthful of regular saline.

Group II-given intravenously with morphine at a dosage of 1 milligramme per kilogramme of body weight.

Group III-given intraperitoneally at a dosage of 62.5 milligrammes per kilogramme of body weight.

Group IV-given intraperitoneally at a dosage of 125 milligrammes per kilogramme of body weight.

Group V-injected intraperitoneally with an extract dose of 250 milligrammes per kilogramme of body weight.

Group VI-injected with 500 milligrammes of neem extract per kilogramme of body weight.

**Extraction procedure**

The preferred method is Soxhlet extraction method for isolation of herbal active constituents. About 500 gm powder (maximum capacity of thimble to hold powder) of dried Stems was accurately weighed and used forextraction. To remove any large or small particles, the powder was run through a 120# mesh sieve. Soxhletex traction and petroleum ether were employed to extract the powder (60-80$^0$c) as a solvent. The extraction was done using above mentioned solvents using increased order of their polarity starting from lowest to highest from Petroleum ether, ethanol. After completion of extraction by Petroleum Ether (as we selected solvent as per their polarity from higher to lower and petroleum ether also used to separate fatty acids from extract) solvent was removed and the diluted extract was dried in the open air to avoid loss of constituents from extract.

**Animals**

Anxiolytic activity-The experiments were conducted using Two- to five-gram male or female Swiss albino mice. The rodents were given rodent pellet diets from Nutrivet Life Sciences in Pune and were kept in a room with regulated temperatures (25 ±1 ° C), humidity (45-65%), and light (12 h light/12 h dark cycle, light at 07:00 a.m.). The animals were allowed to adjust to the laboratory environment for a minimum of 48 hours before to the studies. Pravara Rural College of Pharmacy in Pravaranagar has given its stamp of approval to the study approaches, India, Institutional Animal Ethical Committee.

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(Protocol No.:1942/PO/Re/S/17/CPCSEA/2018/02/12) (Form B [per rule 8(a)]).

**Analgesic activirty-**

We got male Wister albino rats (180-220 g) from the zoo.

Regular laboratory feed and water were provided to the animal's ad libitum, and they resided at room temperature (22-28 ºC) for seven days with a 12-hour light and dark cycle. The cleanliness of our animal house was always a top priority. Institutional Animal Ethics Committee (CPCSEA) approval and protocol adherence ensured the safety and moral operation of the research

**Drugs and chemicals**

The analytical grade solutions utilised in this research were sourced from Merck in India, diazepam injection (Ranbaxy Laboratories Ltd, Baddi, Solan) were purchased and used.

**Pharmacological activities of extract**

**Anxiolytic activity-Elevated plus maze (EPM)**

Modifications have been made to the elevated plus maze (EPM) test involving mice. According to Schmitt and Hiemke (1998), the test primarily modifies behaviours connected to anxiety by combining receptivity with elevations. This theory is based on research on rodent nervousness, which has shown that these animals prefer small, gloomy places and have an irrational fear of both heights and open areas (Bradley et al., 2007). Two open, horizontal arms (30 cm × 5 cm) and two shut down horizontal arms (30 cm × 5 cm × 25 cm) made up the EPM of the mice. The arms were open on top. The labyrinth, constructed of dark-painted wood, stood 45 centimetres above the floor (Lister, 1987). For five minutes, the mouse was watched while its arm was stuck in the raised plus maze's centre opening. The amount of submissions and the amount of time spent working on the open and closed arms submissions were assessed throughout the time frame of the test. When all four paws went into the arm, it was considered an arm entrance. Medications that reduce anxiety specifically lengthen the duration of open-arm exercises and boost the frequency of open-arm entries. The raised plus maze measures locomotor activity as the total amount of arm entrants (the amount of open arm entries + number of closed arm entries; Lister, 1987; de Castro et al., 2007; Raupp et al., 2008), which remained unchanged during the time these effects were detected. The maze was wiped down with a 10% (v/v) ethanol solution with dry towels prior to each experiment.

**Analgesic activity**

Each of the six groups of Wister albino rats was given six animals to test for analgesics efficacy. One of the six groups is given a typical medicine, such as morphine, while the other two get normal saline as a comparison. As a vehicle for testing for substances, this normal saline was utilised. The other four groups of albino rats were given varying amounts of neem oil through intraperitoneal injection: 62.5 mg, 125 mg, 250 mg, and 500 mg/kg body weight.

The experimental pain model assessed the analgesic effects of two drugs—neem extract and morphine—by measuring the tail flick reaction to thermal activation. An analgesiometer, a pharmacological device, was used to track the thermal activation. The top of an analgesiometer, which is an enclosed device, is a nichrome wire. Two points are connected by this nichrome wire. Red light and radiant heat are produced by this nichrome wire whenever the analgesiometer is turned on. The nichrome wire's energy meter was set at 5 amperes, and that's the amount of current that passed through it.

To isolate the sense of radiant warmth, the albino rat had its tail held slowly 3 mm above the nichrome wire, approximately 1.5 cm from the tip. Upon sensing radiant heat, the albino rat detaches its tail from the nichrome wire. The measurement of the time it took for the tail flick to happen was called the tail flick latency (TFL) period. We made sure the albino rats had a basal TFL of 3-5 seconds and a predetermined cut-off time for response of 10 seconds to prevent any tissue injury by adjusting the strength of the nichrome wire heat. Prior to and following medication delivery, the TFL in every animal was monitored and recorded. At various intervals of 30, 60, 90, 120, and 180 minutes following treatment with each dose of medication, the TFL measurement was obtained. The neem extract was delivered intraperitoneally at doses of 62.5, 125, 250, and 500 mg/kg depending on weight.

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## IV. LITERATURE REVIEW

1. **Viola H, *Et al.,*** (1994) examined the process of extracting benzodiazepine receptor ligands from Tilia tomentosa (Tiliaceae) that have been found to be chemically active. Compounds of a flavonoid type are found in tulip species, which are traditionally used medicinally in Latin America as tranquillizers and tranquillizers. By decreasing ambulatory psychomotor activity, these chemicals clearly had anxiolytic effects when given via the abdomen to mice.
2. **Mohammad A. Alzohairy Et al.,** studied the the medicinal function of neem, or Azadirachta indica, and its active ingredients in the treatment and prevention of illness. The study brought attention to several medicinally significant bioactive components of neem, including quercetin, nimbolide, and nimbin. There is evidence that these chemicals have antibacterial, anti-inflammatory, antioxidant, anticancer, and antidiabetic properties. According to the research, neem and its components may have useful properties for the treatment and prevention of numerous ailments, emphasizing the importance of neem in traditional and modern medicine.
3. **Mahmoud, D.A et al** studied the various neem leaf extracts and nimonol's antifungal effects on a number of significant human diseases. Their research demonstrated that neem leaf extracts and nimonol exhibit significant antifungal properties, effectively inhibiting the growth of various pathogenic fungi. This study highlights the promising properties of neem as an all-natural remedy for fungal infections for treating fungal infections in humans.
4. **Nogueira E, *et al.,*** (1998) examined the rat and mouse effects of Rubus brasilensis on anxiety and depression. Thirty minutes prior to behavioural assessment, the extracts were orally given to male Swiss mice and Wistar rats. The research concluded that the ethanolic extract's aqueous and butanolic fractions were ineffective in reducing anxiety. Theoretically, the GABA(A)-benzodiazepine receptor combination may be activated by a liposoluble component with less acute toxicities, which would explain the sedative action.
5. Bhattacharya SK, Satyan KS. described in full the experimental procedures used to evaluate anti-anxiety and other psychotropic drugs in rats. Their work outlines various behavioral tests and protocols by which prospective medicinal substances' anxiolytic properties might be evaluated in rodent models. These methods include:
6. **Schmidt C, *et al.,*** (1998) tested gamma-hydroxybutyrate's (GHB) ability to alleviate anxiety by use of the Elevated Plus Maze (EPM). The benzodiazepine receptor antagonist flumazenil was discovered to counteract the anxiolytic effects of GHB. Flumazenil (10 mg/kg i.p.) counteracted the calming properties of GHB, a neuromodulatory agent in the brain. These findings point to the GABA(A) receptor complex as the site of action for GHB's anxiolytic and, by extension, antidepressant effects benzodiazepines exert their effects.

## V. RESULT AND DISCUSSION

This study used Azadirachta indica bark, which is a member of the Meliaceae family. Investigations into the pharmacognostic, plant chemicals, and pharmacological properties of Azadirachta indica stems and bark were conducted on samples taken from Sangamner in the Ahmednagar district. The botanical samples were verified and confirmed by the BSI in Pune. The fine powder of desired coarseness was obtained by grinding dried plant tissue using an automatic grinder and then passing it through screen no. 60.

**Antianxiety-like activity of extracts from the bark of *Azadirachta indica* A. Juss. Bark**

**Studies on Acute Toxicology:** The hydroethanolic extract was given directly to mice in doses ranging from 50 to 2000 mg/kg. The animals were monitored for noticeable changes in behaviour, including inattention, getting ready, seizures, drowsiness, hypothermia, and death, for 24 hours following drug treatment. The doses that were chosen were 50 mg/kg, 100 mg/kg, and 200 mg/kg, according to their weight.

**Effects of extracts on the elevated plus maze test-**

In a study with mice, diazepam was found to have anxiolytic-like effects on the amount of time invested in the open arm ($P<0.01$ vs. control) when compared to a group of positive controls given 1 mg/kg of diazepam. In Figure 9, we can see that the duration spent in the open arm of the EPM is substantially shortened by the AIHEE extracts at doses of 200 mg/kg ($P<0.01$), 100 mg/kg ($P<0.01$), and 50 mg/kg ($P<0.01$), respectively. According to Figure 10, the amount of time spent in the resolved arm of the EPM was found to be statistically reduced when given diazepam (1 mg/kg; P<

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0.01 vs. control), AIHEE (200 mg/kg; P< 0.01 vs. control), AIHEE (100 mg/kg; P< 0.01 vs. control), and AIHEE (50, 100, and 200 mg/kg; P< 0.01, P< 0.01 and P< 0.01 vs. control accordingly).

**Table No. 2. Effect of *A. indica* bark extract on the way mice act in the elevated plus maze experiment.**

(Values are mean + SE)

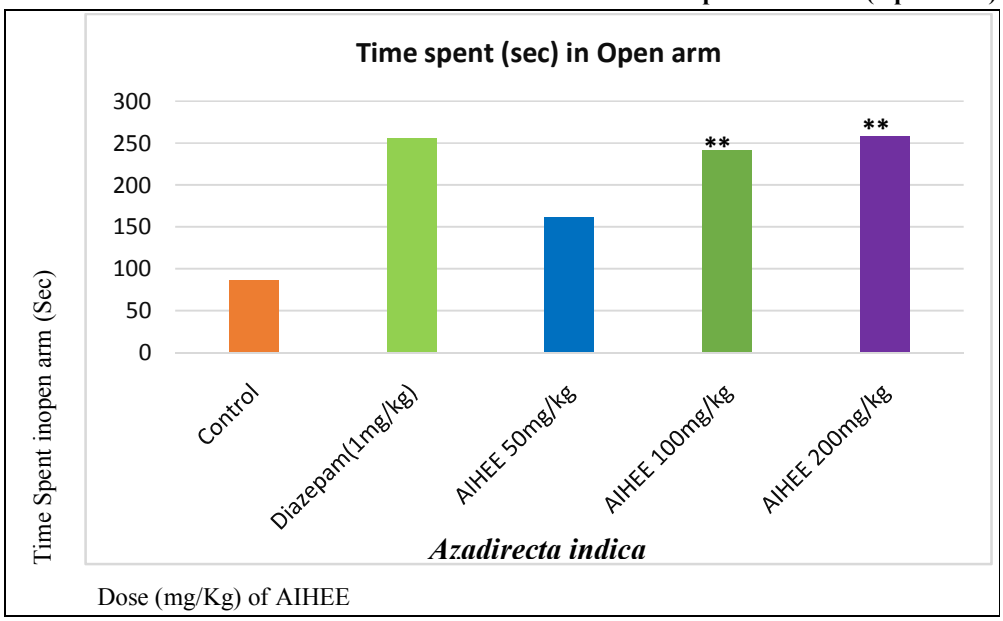
| Groups | No of animals | Time spent (sec) in Open arm |
|---|---|---|
| Control | 06 | 86.83 + 4.976 |
| Diazepam(1mg/kg) | 06 | 256 + 6.077 |
| *A.Indica* Hydroethanolic Extract (mg/kg) | | |
| 50 | 06 | 161.33 + 2.348 |
| 100 | 06 | 241.66 + 3.490 |
| 200 | 06 | 258.16 + 2.688 |

**Table No. -3Effect of *A. indica* bark extract the raised plus maze test for mouse activity (Values are mean + SE)**

| Groups | No of animals | Time spent (sec) in Close arm |
|---|---|---|
| Control | 06 | 213.16 + 4.976 |
| Diazepam(1mg/kg) | 06 | 44 + 6.077 |
| *A.Indica* (mg/kg) | | |
| 50 | 06 | 138.66 + 2.348 |
| 100 | 06 | 58.33 + 3.490 |
| 200 | 06 | 40.66 + 2.578 |

**GRAPHS**

**Graph.01. Effect of *A .indica* bark extract on behaviour of mice in elevated plus maze test. (Open Arm)**




** In contrast to the control group, there is a significant difference (p< 0.01). The values represent the average plus standard deviation of six animals.

Graph.02. Effect of *A. indica* bark take a look at how mice act in a maze with a raised experiment. (Close-Up View)

** In contrast to the control group, there is a significant difference (p< 0.01). Values are given as mean + SD of six animals.

Acute administration of Azadirachta indicaA. Juss. extracted bark to mice: effects on the EPM test. Oral therapies with saline solution as a control, diazepam (DZP1; 1 mg/kg), and AIHEE (50, 100, and 200 mg/kg) were presented as the

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ratio of open arm to closed arm time. Mean ± SEM is the way the results are presented. In comparison to the control group, there were significant differences (**P < 0.01, **P < 0.01, and **P < 0.01).Mice treated with a solution derived from the bark of the Azadirachta indica plant showed considerable reductions in anxiety.Time spent open arms was much longer and time spent closed arms was much shorter after administering extracts at doses of 50, 100, and 200 mg/kg as opposed to the control group. The total amount of entrances on open arms comparing to close arms is considerably increased at doses of 100 and 200 mg/kg as well. The open vs. close arm ratio of entries to total time were also found to be significantly greater for the *A.indica* treated (50,100,200 mg/kg ) groups.

**Effects of extracts on the Analgesic activity-**The research findings tested using the use of the Analysis of Variance (ANOVA) test. The study involved different experimental groups, including a control group administered normal saline, a standard group administered morphine, and groups administered with alcoholic extract of neem at various dose concentrations. Here's a summary of the results:

Group Control: Baseline Tail Flick Latency (TFL) showed not a statistically significant modification in animals given normal saline (control group). This data points to the lack of analgesic action of the saline. The animals in the conventional group, which received morphine via the abdomen (i.p.) at a dose of 1 mg/kg body weight, demonstrated analgesia at different time points. The average group's analgesic effect peaked at 60 minutes' post-administration. The analgesic impact of alcoholic neem powder increased in a dose-dependent manner in animals given varying doses of the extract. After 30–90 minutes after treatment, the Tail Flick Latency (TFL) began to rise, and by 180 minutes, it had reached its basal value. At 60 minutes following delivery, the groups receiving the extract showed the greatest analgesics operation, especially with 250 mg/kg and 500 mg/kg body weight i.p. dosages. To visually compare the results, a bar graph may have been constructed, depicting the analgesic effect of the standard group (morphine) alongside the extract-treated groups at different time intervals. The peak analgesic effect observed in the extract-treated groups, particularly at doses of 250mg/kg and 500mg/kg, may have been highlighted for comparison with the standard group. This research suggests neem's alcohol solution shows substantial analgesic properties, with the peak effect observed at 60 minutes after administration, comparable to the effect of morphine.

**Table No. 4- .Effect of extracts on tail flick latency (TFL) at various time intervals**

| Treated groups | Mean basal TFL (in sec.) | Mean TFL ± SEM (In Sec.) | | | | |
|---|---|---|---|---|---|---|
| | | 30 min. | 60 min. | 90 min. | 120 min. | 180 min. |
| Control group ( normal saline)0.5ml/rat | 3.8 ± 0.22 4.0 ± | 4.0 ± 0.12 | 4.1 ± 0.26 | 4.1 ± 0.23 | 4.0 ± 0.27 | 3.9 ± 0.22 4.0 ± |
| Standard group (morphine) 1mg/kg | 3.7 ± 0.25 | 9.5 ± 0.39 | 10.8 ± 0.35 | 10.4 ± 0.56 | 9.6 ± 0.25 | 5.3 ± 0.18 |
| E 62.5mg/kg | 3.4 ± 0.19 | 4.6 ± 0.23 | 6.6 ± 0.50 | 6.9 ± 0.28 | 5.85 ± 0.47 | 4.0 ± 0.15 |
| E 125mg/kg | 3.5 ± 0.20 | 6.6 ± 0.60 | 8.8 ± 0.40 | 9.1 ± 0.23 | 8.0 ± 0.45 | 4.1 ± 0.24 |
| E 250mg/kg | 3.7 ± 0.40 | 7.7 ± 0.32 | 9.5 ± 0.25 | 9.2 ± 0.22 | 8.5 ± 0.22 | 4.2 ± 0.26 |
| E 500mg/kg | 4.0 ± 0.25 | 8.9 ±0.45 | 10.1 ± 0.21 | 9.4 ± 0.22 | 9.2 ± 0.40 | 4.1 ± 0.24 |

(Statistically significant at P < 0.05 when compared to the control and benchmark groups; values are shown as the mean ± SEM; n = 6).

The findings of the study indicate that neem bark extract possesses analgesic activity, as demonstrated through a dose-dependent study using the heat activation of the albino rat tail flick paradigm. The analgesic efficacy of neem leaf extract was evaluated in comparison to that of morphine, a gold standard medicine, when given at a dosage of 4 mg/kg,

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while a control group received normal saline. Morphine, being an opioid analgesic, acts on supraspinal receptors (μ1, K3, δ1, δ3) and spinal receptors (μ2, K1, δ3). On the other hand, the analgesic effects of a extract of n are thought to be mediated via the opioid receptors that act regionally. In particular, the extract triggers the secretion of indigenous peptides (endorphins) by acting on the periaqueductal grey matter. The endogenous peptides subsequently go down the spinal cord and block the transmission of pain impulses at the dorsal horn terminals.Standard medication morphine showed strong analgesic effects in the laboratory setting when given at a dose of 1 mg/kg body weight, peaking at 60 minutes post-administration and returning to basal Tail Flick Latency (TFL) levels by 180 minutes.In contrast, when neem leaf extract was administered at doses of 250mg/kg and 500mg/kg body weight, the maximum TFL was observed at 60 minutes post-administration. This suggests that the neem leaf extract, like morphine, produced a significant analgesic effect, with the peak effect observed during the identical intervals. In sum, these findings support the notion that neem leaf extract possesses analgesic properties mediated through centrally acting opioid receptors, similar to morphine. Nevertheless, additional investigation may be necessary to establish the precise processes behind the analgesic action of neem leaf extracts and to explore its potential therapeutic applications.

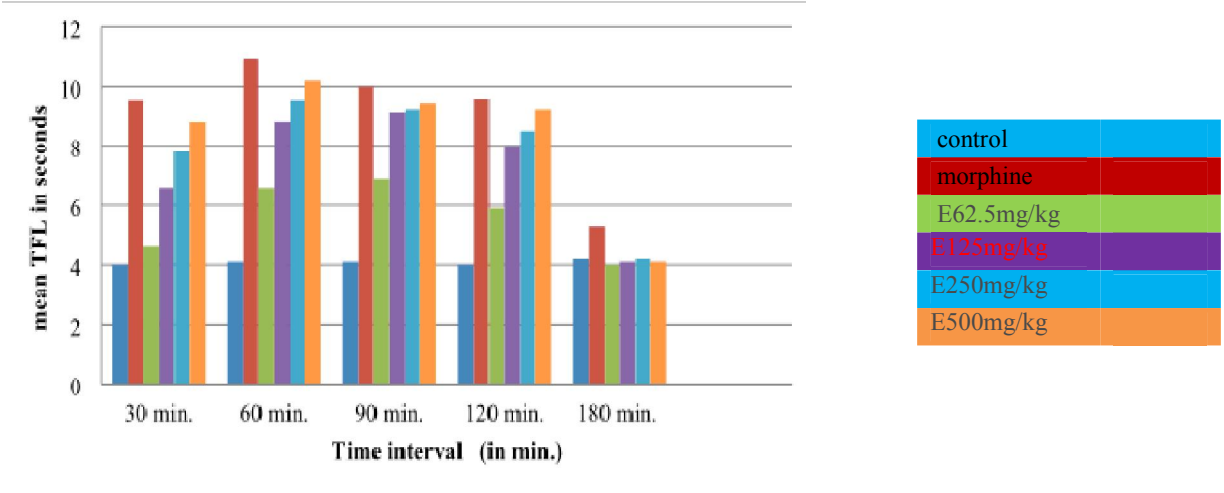


Figure: Comparative impact at various time intervals on the mean TFL (in millilitres)

## VI. CONCLUSION

The hydroethanolic extract obtained from the maceration was evaporated to remove the solvent, leaving behind a concentrated extract. The concentrated extract was then collected for further phytochemical analysis and potential pharmacological testing. This extraction process effectively isolates a wide range of phytochemicals present in the *Azadirachta indica* bark, allowing for subsequent analysis of bioactive substances that aid in the plant's medicinal properties.

The Sangamner area of the Ahmednagar district was surveyed for the fresh bark of the Azadirachta indica Linn (Neem). The botanical samples were verified and confirmed by the BSI in Pune. The dried plant stems bark was reduced to small particle size by milling and then subjected to de fatationby petroleum ether as solvent. Drawing on research Bark tannins include gallic acid, (+) gallocatechin, (-) epicatechin, (+) catechin, and epigallocatechin; of these, gallic acid (1), (-) epicatechin (2), and catechin (3) are the most important for preventing turned on human oligomorphonuclear neutrophils (PMNs) from generating the process of chem suggesting that these substances prevent PMNs from undergoing an oxidative surge during swelling bark of *Azadirachta indica* as active constituents.

In the Pharmacognostic study i.e. macroscopy, physical standardization and powder characteristics of stem bark of plant were studied. Macroscopy of stem shows presence of colour as rusted grey, odour as characteristic. The stem bark of plant was extracted by using Soxhlet extraction method and solvent used as petroleum ether, Ethanol. The percentage yield of petroleum ether, hydroethanol extract was found to be 9.5 %,16.7 % respectively. Preliminary phytochemical screening of extract was done.Some part of extract was then used to study pharmacological screening of anti-anxiety

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activity. From the study of phytochemical and pharmacological investigation of Azadirachta indica bark it was found that different chemicals are present as glycosides, alkaloids, tannins, fats, steroids, proteins, etc. Azadirachta indica stem bark supports the elimination of related anxiety associated materials. Therefore, drawing conclusions from this research suggests that Azadirachta indica hydroethanolice etract were found to be with good antianxiety like activity in elevated plus maze model.

Furthermore, this current study confirms the conventional utilisation of Azadirachta indica bark as a pain reliever by showing that its hydroethanolic determine has noteworthy analgesic properties for various dose amounts, with the greatest analgesic movement observed at a 60-minute time interval. More research is required, nevertheless, to determine how precisely it exerts its analgesic effects.

## VII. ACKNOWLEDGEMENT

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