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Anxiolytic Prospective of Limonene Terpenoids-A Preclinical Investigation

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ABSTRACT

Anxiety is a common and often disabling mental health issue affecting millions globally. Traditional plant-based remedies have gained attention as safer alternatives to conventional medications. In this study, we investigated the anti-anxiety potential of biosynthesized silver nanoparticles (Ag-NPs) derived from Abies pindrow Royle leaf extracts limonene. Using a green synthesis method, Ag-NPs were prepared and characterized. Their effects were evaluated in a rodent model of anxiety using behavioral tests (Elevated Plus Maze), biochemical markers of oxidative stress, and brain histology. The results demonstrated that Abies pindrow- terpenoids derived Ag-NPs significantly reduced anxiety-like behaviors, lowered oxidative stress levels, and showed protective effects on brain tissues. These findings suggest that green-synthesized Ag-NPs from Abies pindrow Royle may represent a promising natural therapeutic approach for managing anxiety.

KEYWORDS: Anxiety, Abies pindrow Royle, silver nanoparticles, green synthesis, oxidative stress, elevated plus maze

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INTRODUCTION

Anxiety is a natural emotion, but when it becomes excessive and persistent, it leads to disorders that impact daily life [1]. While conventional treatments like benzodiazepines and antidepressants are effective, they can cause side effects and dependence. Therefore, researchers are exploring plant-based alternatives [2]. *Abies pindrow Royle*, commonly known as the Himalayan Silver Fir, has a long history in traditional medicine for treating respiratory issues, inflammation, and nervous [3] system disorders. Recent studies have suggested that extracts from this plant possess antioxidant and anti-inflammatory properties, which may help in reducing anxiety [4]. With advancements in nanotechnology, green synthesis using plant extracts has emerged as an eco-friendly method to produce nanoparticles. Silver nanoparticles (Ag-NPs) are known for their antioxidant, antimicrobial, and potential [5] neuroprotective effects. Thus, combining Abies pindrow extracts with silver nanoparticles could offer a novel approach to anxiety treatment [6].

MATERIALS AND METHODS

Plant Material Collection and Extraction

Leaves of Abies pindrow Royle were collected, dried, and processed to prepare extracts. Phytochemical screening confirmed the presence of key bioactive compounds such as flavonoids and phenolic compounds.

Green Synthesis of Silver Nanoparticles

Silver nanoparticles were synthesized using the plant extract as a reducing and stabilizing agent. The synthesis conditions included

controlled concentration, temperature, and stirring speed to ensure uniform particle formation. Recent advancements in nanotechnology have highlighted the potential of green synthesis methods for producing silver nanoparticles (Ag-NPs), which offer a safer and more sustainable alternative to conventional chemical processes. One promising approach involves the use of plant-based materials rich in natural reducing agents [7]. Among these, *Abies pindrow Royle*, a terpenoids Himalayan conifer known for its medicinal properties, has emerged as a valuable source for the eco-friendly synthesis of (Ag-NPs). Extracts from this plant contain various bioactive compounds — including phenolics, flavonoids, and terpenoids — that not only facilitate the formation of nanoparticles but also enhance their biological functionality.

Table No. 01: Variables used for Green Synthesis of silver Nano particles

Sr. no.	***Abbies p. royle* Extract conc. (mg/ml)**	**Conc. Og AgNO3 solution**	**Temperature (°C)**	**Stirring speed (rpm)**
1.	6	1mMAgNO3	35	400
2.	8		40	500
3.	10		50	600
4.	12		60	700
5.	14		70	800

Animal Model and Experimental Design

Rodents were used as the experimental model to evaluate anxiety-related behaviors. The animals were divided into groups: Normal group, control group, standard drug group and Ag-NP-treated groups.

Table. No. 01: Experimental protocol. The animals were often separated into six groups, with six animals in each group.

Groups	**Pharmacological Interventions**	**Dose(mg/kg)**	**Route (p.o.)**	**No. of animals**
Group 1	Normal control Group	Water	Oral	5
Group 2	Disease Control Group	EPM	-	5
Group 3	Test group-I	50	Oral	5
Group 4	Test group-II	100	Oral	5
Group 5	Test group-III	200	Oral	5
Group 6	Standard Group (Diazepam)	2	Oral	5

Behavioral Assessment

The Elevated Plus Maze (EPM), Locomotor activity and light/Dark box was used to assess anxiety. The test measured time spent in open and closed arms, and the number of entries into each [8].

Figure No. 1: Elevated plus maze

Biochemical Analysis

Brain tissues were analyzed for oxidative stress markers, including glutathione (GSH) levels and thiobarbituric acid-reactive substances (TBARS). Anxiety was experimentally induced in mice using the Elevated Plus Maze (EPM) model to assess oxidative stress by measuring TBARS (Thiobarbituric Acid Reactive Substances) levels in brain tissue. TBARS, a key indicator of lipid peroxidation, was significantly elevated in the anxiety-induced group compared to the control, reflecting increased oxidative stress. Treatment with the standard anxiolytic drug diazepam (2 mg/kg) significantly reduced TBARS levels, indicating its antioxidant and neuroprotective effects. Similarly, administration of *Abies pindrow Royle* extract at varying doses (50, 100, and 200 mg/kg) produced a dose-dependent reduction in TBARS levels. TBARS readings were similar to those of the diazepam-treated group, indicating that the 200 mg/kg dose was especially effective. Abies pindrow Royle may be used therapeutically to treat anxiety disorders because of its strong antioxidant activity and potential to provide neuroprotection against oxidative damage brought on by anxiety [9].

Glutathione (GSH), a significant natural antioxidant, is essential for preventing oxidative damage to brain tissue. In this study, mice were given the Elevated Plus Maze (EPM) model to elicit anxiety. This led to a large drop in brain GSH levels, suggesting that anxiety is related with increased oxidative stress. GSH levels were markedly restored after treatment with the common anxiolytic medication diazepam (2 mg/kg), indicating its neuroprotective and antioxidant properties. Likewise, GSH levels increased in a dose-dependent manner when 50, 100, and 200 mg/kg of *Abies pindrow Royle* extract were given. The greatest rise was seen at 200 mg/kg, which almost brought GSH levels back to normal. Abies pindrow Royle's potent antioxidant properties can successfully lower oxidative stress by raising GSH levels in the brain. Consequently, providing neuroprotection in situations where anxiety is present.

Histopathological Examination

Brain sections were examined under a microscope to assess structural changes and neuronal protection. The histological analysis of the brains in the various groups will probably show clear alterations associated with anxiety and the results of therapy. Brain tissue from the normal group should show no symptoms of inflammation or degeneration and a healthy neuronal architecture [10]. As a result of anxiety-induced oxidative stress and neuroinflammation, the raised plus maze disease group may exhibit gliosis, increased apoptosis, and neuronal damage. Diazepam treatment (2 mg/kg) is anticipated to promote neuroprotection by lowering glial activity and maintaining neuronal structures. While the 200 mg/kg dose should exhibit stronger neuroprotective effects, akin to diazepam, with restored neuronal integrity, reduced apoptosis, and decreased neuroinflammation, the 100 mg/kg dose may display partial protection in the *Abbies pindrow royle* groups with some reduction in neuronal damage and gliosis. The findings imply that *Abbies pindrow royle* limonene has anxiolytic and neuroprotective effects that are dose-dependent and may be mediated by its antioxidant qualities [11] [12].

RESULTS

Behavioral Outcomes

Animals treated with Abies pindrow-derived Ag-NPs spent significantly more time in open arms of the EPM, locomotor activity and light/dark box indicating reduced anxiety levels, comparable to the diazepam group.

Table No. 02: Effect of *Abbies pindrow royle* (50,100,150 mg/kg) on light dark box activity (sec and minutes), locomotor activity in Actophotometer where a vs normal control; b vs disease control and Values are reported as $p<0.01$ and mean± S.D.

Groups	Time spent in arms (s)	Time spent in Closed Arms (s)	Entries in Open Arms	Entries in Closed Arms	Locomotor activity (count/per)
Group-1 Normal Control	150 ± 12	250 ± 15	6 ± 1	20 ± 2	216.7±1.083
Group-2 Disease (Anxiety Model)	70 ± 8 [a]	330 ± 12 [a]	3 ± 1 [a]	10 ± 3 [a]	97.2±0.486[a]
Group-3 Diazepam (2mg/kg)	200 ± 15 [b]	200 ± 10 [b]	8 ± 2 [b]	30 ± 2 [b]	158± 0.79 [b]
Group-4 *Abbies pindrow royle (50mg/kg)*	160 ± 12 [b, c 1]	240 ± 12 [b, c 1]	6 ± 1 [b, c 1]	22 ± 2 [b, c 1]	160±0.87 [b, c1]
Group-5 *Abbies pindrow royle (1o0mg/kg)*	180 ± 14 [b, c 2]	220 ± 11 [b, c 2]	7 ± 1 [b, c 2]	28 ± 2 [b, c 2]	162± 0.81 [b, c 2]

Group-6 *Abbies pindrow royle (200mg/kg)*	185±16 b, c 3	230±15 b, c 3	9±1 b, c 3	24±3 b, c 3	174±0.92 b, c 3

Table No. 03: Effect of Abbies pindrow royle (50,100,150 mg/kg) on elevated plus maze (sec and minutes) where a vs normal control; b vs disease control and Values are reported as p<0.01 and mean± S.D.

Group	Time in open Arms (s)	Time in Closed Arms (s)	Entries in Open Arms	Entries in Closed Arms
Normal Control	180 ± 10	120 ± 9	8 ± 1	10 ± 2
Disease (Anxiety Model)	60 ± 8 a	240 ± 12 a	3 ± 1 a	15 ± 3 a
Diazepam (2mg/kg)	200 ± 12 b	100 ± 10 b	9 ± 1 b	8 ± 2 b
Abbies pindrow royle (50mg/kg)	100 ± 10 b, c 1	200 ± 12 b, c 1	5 ± 1 b, c 1	11 ± 2 b, c1
Abbies pindrow royle (100mg/kg)	140 ± 12 b, c 2	160 ± 10 b, c 2	6 ± 1 b, c 2	9 ± 2 b, c 2
Abbies pindrow royle (200mg/kg)	150 ± 12 b, c 3	180 ± 12 b, c 3	7 ± 1 b, c3	8 ± 2 b, c 3

Oxidative Stress Markers

Treatment with Ag-NPs increased brain GSH levels and decreased TBARS, reflecting reduced oxidative stress.

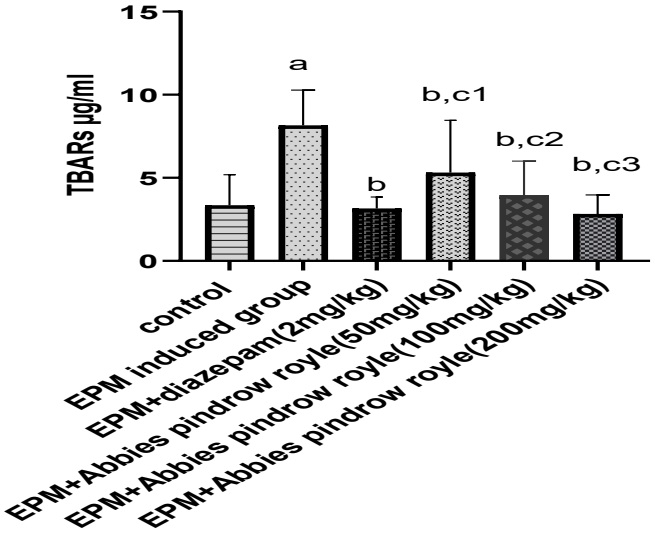

Figure no 2: TBARs Estimation in different Grouped Animal.

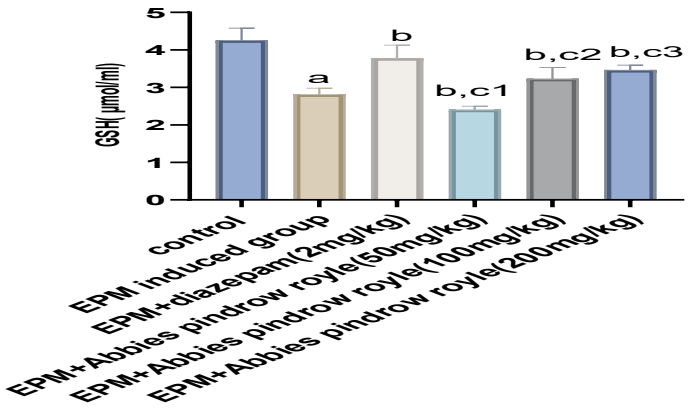

Figure No. 3: GSH Estimation in different Grouped Animal.

Histological Findings

Histopathological analysis showed improved neuronal integrity and reduced signs of damage in the Ag-NP-treated group [13][14].

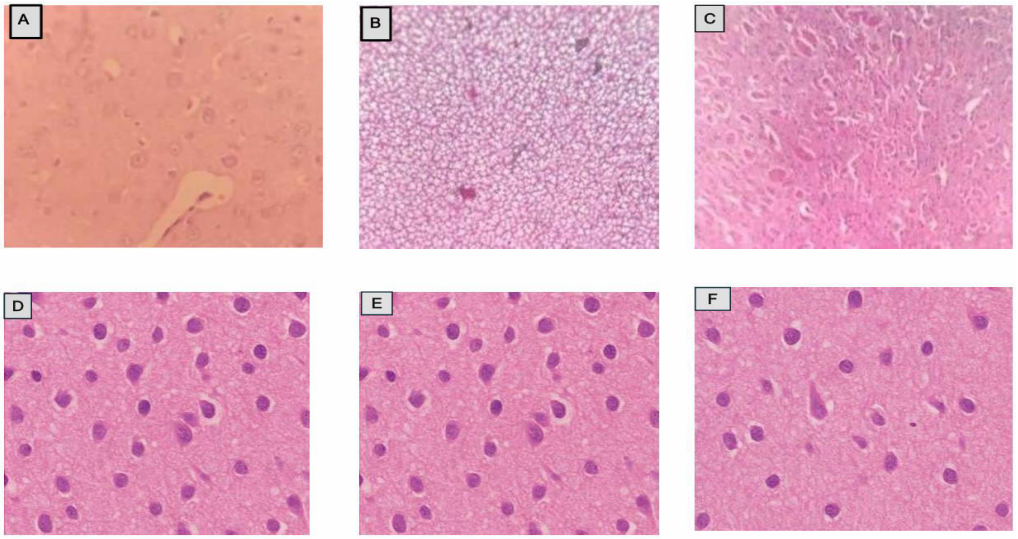

Figure No. 2: A. Normal Control. B Disease Control (EPM). C Standard group (Diazepam). D Treatment Abbies pindrow royle (50mg/kg). E Treatment Abbies pindrow royle (100mg/kg) F. Treatment Abbies pindrow royle (200mg/kg)

DISCUSSION

The study demonstrates that silver nanoparticles synthesized from *Abies pindrow Royle* terpenoids extracts have strong anti-anxiety effects [15]. The behavioral improvements were supported by reduced oxidative stress and preserved brain tissue structure. The observed benefits may be attributed to the high antioxidant content of *Abies pindrow*, which helps counteract reactive oxygen species implicated in anxiety pathophysiology. Additionally, the use of green synthesis avoids toxic chemicals, making it a safer and environmentally friendly approach. These findings open possibilities for developing natural, nanotechnology-based therapies for anxiety. However, further studies including detailed molecular analyses and human clinical trials are needed to confirm these effects.

CONCLUSION

Abies pindrow Royle terpenoids-derived green-synthesized silver nanoparticles significantly alleviated anxiety-like behavior and oxidative stress in rodents. This formulation will offer more site specific, effective, more targeted and will react favorably to the body. This suggests a promising role for these nanoparticles as a natural anti-anxiety therapy.

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