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**EVALUATION OF ANTIDEPRESSANT ACTIVITY OF  
AQUEOUS ROOT EXTRACT OF ARNEBIA BENTHAMII IN  
RATS**

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**Abstract:**

**Objective:** Depression is an etiologically heterogeneous group of brain disorders characterized by a wide range of symptoms that reflect alterations in psychomotor cognitive and emotional processes. Arnebia benthamii is a high value medicinal plant rank second in the list of medicinal plant prioritized for Western Himalaya. The roots have antihelmentic, antipyretic, antiseptic, antimicrobial and antioxidant properties. The present study was designed to evaluate the antidepressant activity of aqueous root extract of Arnebia benthamii in rats through behavioral assessment such as Force swim test and Tail suspension test and biochemical assessment such as superoxide dismutase, nitrite level, brain glutathione and lipid peroxidation in the rat brain.

**Method:** The test drug was administered orally in three doses (75, 150 & 300 mg/kg p.o) for a period of 14 days. Imipramine (standard), 10 mg/kg p.o, was used as a standard treatment. Forced swim test and tail suspension test were used to assess antidepressant activity. On the 14<sup>th</sup> day, through behavioral testing, effect of the drug was assessed on reduced glutathione, lipid peroxidation, superoxide dismutase and nitrite in the rat brain tissue.

**Results:** The aqueous root extract of Arnebia benthamii (75, 150 & 300 mg/kg) showed significant reduction in immobility time in forced swim test and tail suspension test. Aqueous root extract of Arnebia benthamii significantly increased brain glutathione level, SOD level as compared to the control group and decreased the lipid peroxidation and nitrite as compared to the control group.

**Conclusion:** In the present study the aqueous root extract of Arnebia benthamii shows promising effect of antidepressant activity in rats. Arnebia benthamii which possess antioxidant property have shown high therapeutic value in managing various disorders such as depression induced by force swim test and tail suspension test.

**Key words:** Arnebia benthamii, force swim test, tail suspension test, Imipramine and antioxidant.

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## INTRODUCTION:

### Depression

Depression is an etiologically heterogeneous group of brain disorders characterized by a wide range of symptoms that reflect alterations in psychomotor cognitive and emotional processes [1]. Depression is induced by chronic stress and is one of the main causes even though the mechanism of stimulating depression is not clearly established [2]. Depression is an occurring persistent and potentially debilitating form of psychiatric disorders. It occurs in persons of all gender with no age limit and backgrounds. Although sadness is usually a normal human experience, clinical depression differs both in duration and severity. It is more common in female than male. Any form of stressful life event is considered as the initial sign of depression, thereby depression is often thought as a stress related disorder [3]. Pathogenesis of depression is contributed by the experience of human stress, and it may also play in the seriousness and occurring again of this debilitating illness [4]. As claimed by the World Health report, approximately 450 million people endure from behavioral or mental disorder. This comes to be 12.3% of the worldwide burden of disease and will rise to 15% by 2020[5]. It has suggested that it will be the second major cause of death by the year 2020 because of its high prevalence of suicide in depressed patients coupled with complication arising from stress and its effects on the cardiovascular system. Depression is associated with a serious impairment of marital, occupational functioning and social as well as prominent personal and interpersonal distress [6]. *Arnebia benthamii* a high value medicinal plant rank second in the list of medicinal plant prioritized for Western Himalaya and also figures among the 59 medicinal plants prioritized for conservation due to high extinction threat. The roots yield a red pigment, shikonin and alkanin a lipophilic red pigment which is the main active constituent of this plant and are responsible for its color and therapeutic efficacy. The roots have antihelmenthic, antipyretic, antiseptic antimicrobial and antioxidant properties. The plant also possesses stimulant, tonic, diuretic, and expectorant properties. The flowering shoots are used in preparation of jam and sherbet (syrup) are useful in various diseases of throat, fever, tongue and cardiac disorders. Although in folklore medicine *Arnebia benthamii* has been ascribed with the beneficial medicinal properties. Its consumption should be completely avoided by patients already suffering from any liver disorder as it can cause significant hepatotoxicity[7, 8, 9].

### Chemical constituents of *Arnebia benthamii*

The active constituents of *Arnebia benthamii* are benzoquinones, napthaquinones, triterpenoids, alkaloids, steroids and flavonoids. The other important active constituents of *Arnebia benthamii* are hoslundal, artemidiol, shinkonin, ganoderiol and 2-hexaprenyl-6-hydroxyphenol were found to be of immense importance in curing many disease and disorder in humans and are recommended as an antioxidants. The dry plant yields essential oil Arnebinus [9].

## MATERIALS AND METHODS:

### Plant material

The plant *Arnebia benthamii* was collected from Uttarkashi, Uttarakhand.

### Animals

Wistar albino rats (200-250g) were procured from departmental animal house of Shri Guru Ram Rai Institute of Technology and Science, Patel Nagar, Dehradun. Animals were acclimatized in the animal house facility and housed in polypropylene cages with husk bedding, under 12:12 light dark cycle at  $25\pm5^{\circ}\text{C}$ , and were fed with standard commercial pellet and water *ad libitum*.

### Authentication

The plant has been authenticated from Botanical Survey of India (BSI), Dehradun, Uttarakhand

### Preparation of extract

100g of dried plant powder was added to glass flask containing one liter of water. This was followed by continuous stirring for four days accompanied with mild heating. The solution was then filtered to remove any un-dissolved plant material. The clear aqueous solution obtained after filtration, containing the dissolved plant components, was subjected to drying components and stored. The amount of plant extract obtained on an average was 10g/l[10].

### Experimental Protocol

In this study six (n=6) groups of albino Wistar rats were employed

Group I: Control group, received normal saline 1ml/kg p.o for 14 days

Group II: Standard, received imipramine- 10 mg/kg p.o for 14 days

Group III: Aqueous root extract *Arnebia benthamii* (75mg/kg p.o) was administered for 14 days

Group IV: Aqueous root extract *Arnebia benthamii*(150mg/kg p.o) was administered for 14 days

Group V: Aqueous root extract *Arnebia benthamii*(300mg/kg p.o) was administered for 14 days

#### Evaluation of antidepressant activity

##### Forced swim test

Forced swim test by Porsolt et al. Rats were individually forced to swim in an open cylindrical container (diameter 18cm, height 40cm) containing 15 cm of water at 25° C ± 1°C .Treatment was given 60 min prior to study as described by study design. All animals were forced to swim for 6 min and the duration of immobility was observed and measured during the final 4 min interval of test. Each animal was judged to be immobile when it ceased struggling and remained floating motionless in the water, making only those movements to keep its head above water. A decrease in the duration of immobility is indicative of an antidepressant like effect [11].

##### Tail suspension test

Tail suspension test by Steru et al. Treatment was given 60 min prior to study as described by study design. Rats were suspended on the edge of table, 50cm above the floor, with the help of adhesive taped placed approximately 1cm from the tip of the tail. The total duration of immobility induced by tail suspension was recorded during a 6min of the 10 min period. Animal were considered to be immobile when it did not show any movement of the body, hanged passively and completely motionless [12].

#### Analysis of biochemical parameters

On the 14th day of dosing of all groups, for the biochemical analysis, animals were sacrificed by anaesthetizing with diethyl ether followed by cervical dislocation, immediately after behavioral assessment with forced swim test and tail suspension test for estimation of brain glutathione (GSH), superoxide dismutase, protein, lipid peroxidation (TBARS) and nitrite oxide.

#### Preparation of brain homogenate

All the animals (including control group) were sacrificed by anaesthetizing with diethyl ether followed by decapitation and brain were removed, and rinsed with ice cold saline (isotonic). Brain tissue samples were homogenized in 0.9% NaCl by using tissue homogenizer [13].

#### Estimation of brain glutathione

To 2 mL of 10% of homogenate, which was prepared in sodium chloride solution, 2.5 mL of 0.02M EDTA was added and shaken vigorously. To

2 mL of this mixture 4 mL of cold distilled water and 1 mL of 50% trichloroacetic acid were added and shaken for 10 min. Thereafter, the content were centrifuged at 3000 x g for 15 min following centrifugation, 2 mL of the supernatant was mixed with 0.4M tris buffer (pH 8.9). The whole solution was mixed well and 0.1 mL of 0.01M DTNB was added, the absorbance was read within 5 min of addition of DTNB at 412 nm against reagent blank with no homogenate. For blank reading, the homogenate was substituted by 2 mL of distilled water. The amount of glutathione in tissue was expressed as µmol/g of tissue (Sedlak & Lindsay, 1968 [14].

#### Estimation of lipid peroxidation (TBARS)

Two milliliter of suspension medium was taken from 10% of tissue homogenate. To this, 2 mL of 30% of trichloroacetic acid was added, followed by 2 mL of 0.8% thiobarbituric acid (TBA) reagent. The tubes were covered with aluminum foil and kept in shaking water bath for half an hour at 80°C after half an hour; the tubes were taken out and kept in ice cold water for half an hour. There were then centrifuged at 3000 x g for 15 min. The absorbance of the supernatant was read at 535 nm at room temperature against appropriate blank. Blank consist of 2 mL distilled water, 2 mL of 30% TCA and 2 mL of 0.8% TBA. The content of malonaldehyde (MDA), expressed as n moles formed per milligram of protein in the tissue (Ohkawa et al., 1979) [15].

#### Estimation of superoxide dismutase

Superoxide dismutase (SOD) activity was assayed by the method of Kono. The assay system consisted of EDTA 0.1mM, sodium carbonate 50mM and 96mM of nitro blue tetrazolium (NBT). In the cuvette, 2mL of the above mixture, 0.05mL of hydroxylamine and 0.05mL of the supernatant were added and the autoxidation of hydroxylamine was measured for 2 minutes at 30 second interval by measuring the absorbance at 560nm using Perkin Elmer Lambda 20 spectrophotometer [16].

**Estimation of total protein** – Total protein was done according to the method of Lowry OH , et al 1951. Total amount of brain total protein was representing in mg [17].

#### Brain nitrite estimation

Brain nitrite level was estimated using Greiss reagent and served as an indicator of nitric oxide production. A measure of 500µl of Greiss reagent (1:1 solution of 1% sulfanilamide in 5% phosphoric acid and 0.1% naphthylamine diamine

dihydrochloric acid in water) was added to 100 $\mu$ l of postmitochondrial supernatant and absorbance was then measured at 546 nm. Nitrite concentration was calculated by using a standard curve of sodium nitrite (Green et al. 1982) [18].

#### **Statistical Analysis**

The statistical analysis would be carried out using Graph Pad Prism 7 software. All values were presented as Mean  $\pm$  SEM. Multiple comparisons between different groups were performed using Analysis of Variance (ANOVA) followed by Dunnet's multiple comparison tests.

#### **RESULTS:**

##### **Pharmacological studies**

###### **Behavioral evaluation**

###### **Effect of aqueous root extract of *Arnebia benthamii* (AB) on reduction of immobility period in rats in forced swim test (FST)**

Administration of aqueous root extract of *Arnebia benthamii* (75, 150 and 300 mg/kg) significantly decreased the immobility period in forced swim test in as compared to the control. The higher dose of aqueous root extract of *Arnebia benthamii* (150 and 300 mg/kg) caused a significant reduction in immobility period as compared to control and was more effective than the lower dose as shown in fig.1.

###### **Effect of aqueous root extract of *Arnebia benthamii* (AB) on reduction of immobility period in tail suspension test (TST)**

Administration of aqueous root extract of *Arnebia benthamii* (75, 150 and 300 mg/kg) significantly decreased the immobility period in tail suspension test as compared to the control. The effect of aqueous root extract of *Arnebia benthamii* 75mg/kg was comparable to control group in reducing immobility period. The higher dose of aqueous root extract of *Arnebia benthamii* (150 and 300 mg/kg) caused a significant reduction in immobility period

as compared to control and was more effective than the lower as shown in fig 2.

###### **Biochemical estimation**

###### **Effect of aqueous root extract of *Arnebia benthamii* (AB) on brain glutathione (GSH) of rat induced by forced swim test and tail suspension test**

Administration of aqueous root extract of *Arnebia benthamii* (75, 150 and 300 mg/kg) significantly increased brain glutathione level as compared to control group. However the higher dose of AB (150mg/kg &300 mg/kg) is more effective as shown in fig.3

###### **Effect of aqueous root extract of *Arnebia benthamii* AB on superoxide dismutase (SOD) in rat induced by forced swim test and tail suspension test**

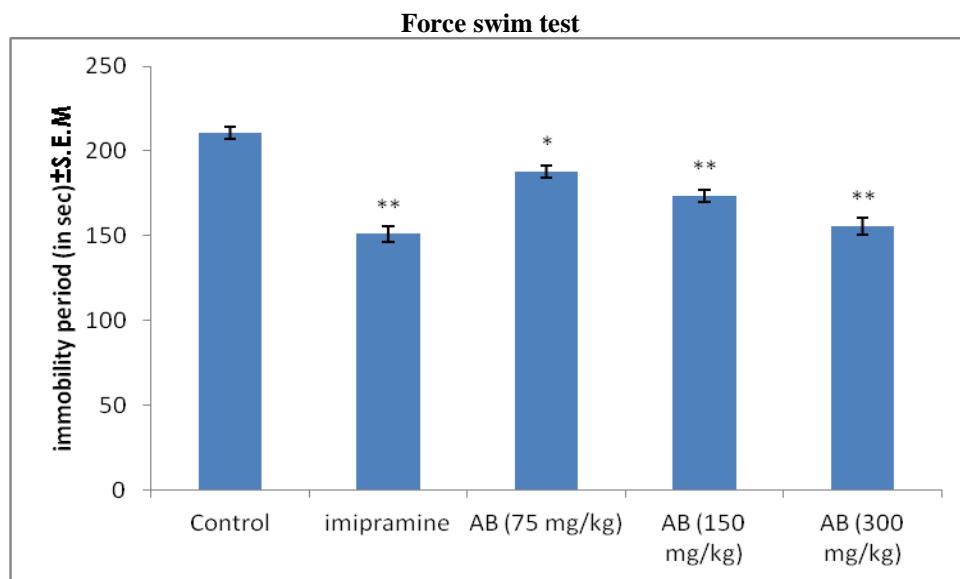
Administration of aqueous root extract of *Arnebia benthamii* (75, 150 and 300 mg/kg) significantly increased superoxide dismutase level as compared to control group. The higher dose of AB (150 &300 mg/kg) is more effective as shown in fig.4.

###### **Effect of aqueous root extract of *Arnebia benthamii* (AB) on lipid peroxidation (TBARS) of rat brain induced by forced swim test and tail suspension test**

Treatment with aqueous root extract of *Arnebia benthamii* (75, 150 and 300 mg/kg) significantly decreased the level of lipid peroxidation as compared to control group. The higher dose of AB (150 &300 mg/kg) is more effective as shown in fig.5.

###### **Effect of aqueous root extract of *Arnebia benthamii* (AB) on nitrite of rat brain induced by forced swim test and tail suspension test**

Treatment with aqueous root extract of *Arnebia benthamii* (75, 150 and 300 mg/kg) significantly decreased the level of nitrite as compared to control group. The higher dose of AB (150 & 300 mg/kg) is more effective as shown in fig. 6

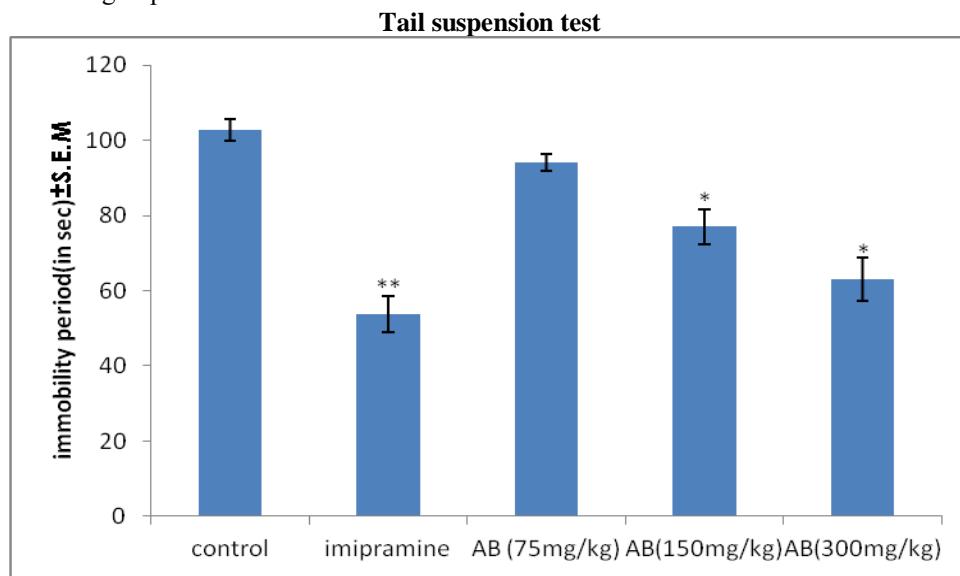


**Fig 1: Effect of aqueous root extract of *Arnebia benthamii* on immobility period in forced swim test.**

**Control** indicates administration of normal saline for 14 days, **Imipramine** indicates administration of Imipramine 10 mg/kg for 14 days, **AB (75mg/kg)** indicates administration of 75mg/kg aqueous root extract of *Arnebia benthamii* for 14 days, **AB (150mg/kg)** indicates administration of 150mg/kg aqueous root extract of *Arnebia benthamii* for 14 days and **AB (300mg/kg)** indicates administration of 300mg/kg aqueous root extract of *Arnebia benthamii* for 14 days.

\*\* =  $p \leq 0.001$  vs control group

\* =  $p \leq 0.01$  vs control group



**Fig 2: Effect of aqueous root extract of AB on immobility period in tail suspension test in rats.**

**Control** indicates administration of normal saline for 14 days, **Imipramine** indicates administration of Imipramine 10 mg/kg for 14 days, **AB (75mg/kg)** indicates administration of 75mg/kg aqueous root extract of *Arnebia benthamii* for 14 days, **AB (150mg/kg)** indicates administration of 150mg/kg aqueous root extract of *Arnebia benthamii* for 14 days and **AB (300mg/kg)** indicates administration of 300mg/kg aqueous root extract of *Arnebia benthamii* 14 days.

\*\* =  $p \leq 0.001$  vs control group

\* =  $p \leq 0.01$  vs control group

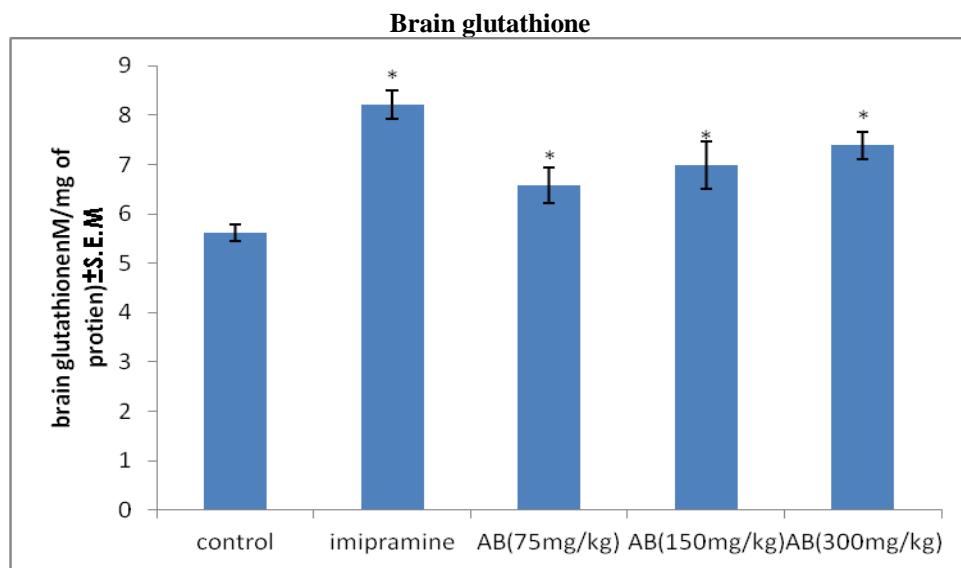


Fig 3: Effect of aqueous root extract of AB on brain glutathione in rats.

**Control** indicates administration of normal saline for 14 days, **Imipramine** indicates administration of Imipramine 10 mg/kg for 14 days, **AB (75mg/kg)** indicates administration of 75mg/kg aqueous root extract of *Arnebia benthamii* for 14 days, **AB (150mg/kg)** indicates administration of 150mg/kg aqueous root extract of *Arnebia benthamii* for 14 days and **AB (300mg/kg)** indicates administration of 300mg/kg aqueous root extract of *Arnebia benthamii* for 14 days.

\*= p≤0.01 vs control group

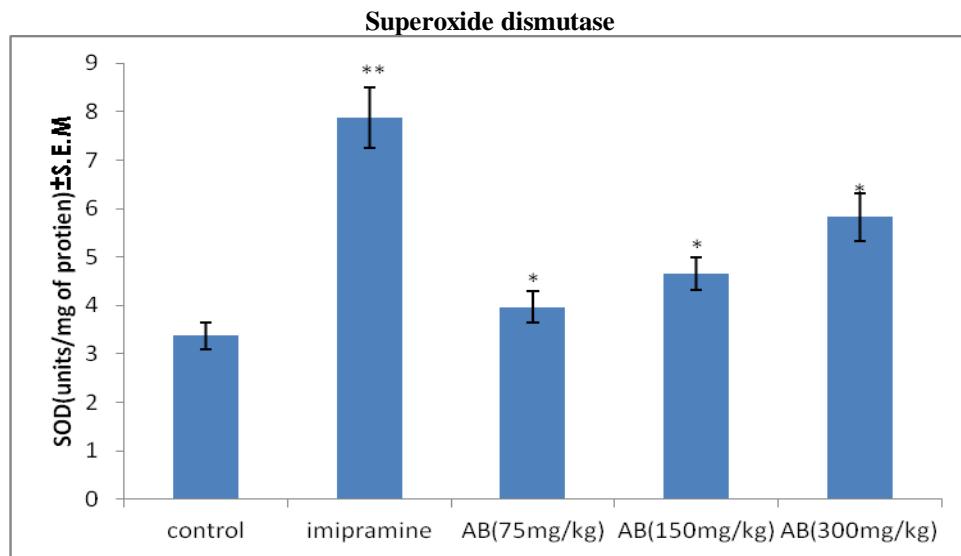
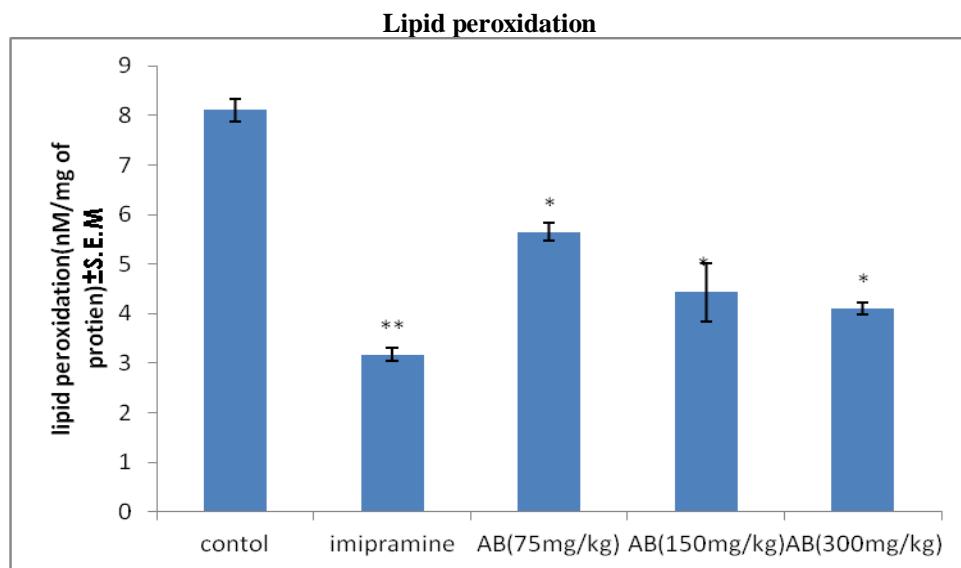


Fig 4: Effect of aqueous root extract of AB on superoxide dismutase in rats.

**Control** indicates administration of normal saline for 14 days, **Imipramine** indicates administration of Imipramine 10 mg/kg for 14 days, **AB (75mg/kg)** indicates administration of 75mg/kg aqueous root extract of *Arnebia benthamii* for 14 days, **AB (150mg/kg)** indicates administration of 150mg/kg aqueous root extract of *Arnebia benthamii* for 14 days and **AB (300mg/kg)** indicates administration of 300mg/kg aqueous root extract of *Arnebia benthamii* for 14 days.

\*\*= p≤ 0.001 vs control group

\*= p≤0.01 vs control group

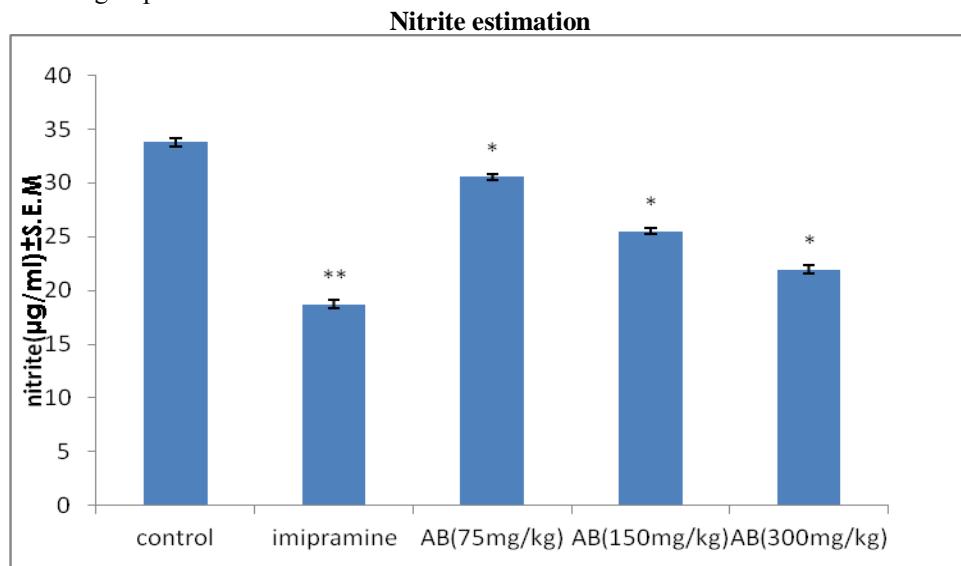


**Fig 5: Effect of aqueous root extract of AB on brain lipid peroxidation in rats.**

**Control** indicates administration of normal saline for 14 days, **Imipramine** indicates administration of Imipramine 10 mg/kg for 14 days, **AB (75mg/kg)** indicates administration of 75mg/kg aqueous root extract of *Arnebia benthamii* for 14 days, **AB (150mg/kg)** indicates administration of 150mg/kg aqueous root extract of *Arnebia benthamii* for 14 days and **AB (300mg/kg)** indicates administration of 300mg/kg aqueous root extract of *Arnebia benthamii* for 14 days.

\* \*\*= p≤ 0.001 vs control group

\*= p≤0.01 vs control group



**Fig 6: Effect of aqueous root extract of *Arnebia benthamii* on brain nitrite in rats.**

**Control** indicates administration of normal saline for 14 days, **Imipramine** indicates administration of Imipramine 10 mg/kg for 14 days, **AB (75mg/kg)** indicates administration of 75mg/kg aqueous root extract of *Arnebia benthamii* for 14 days, **AB(150mg/kg)** indicates administration of 150mg/kg aqueous root extract of *Arnebia benthamii* for 14 days and **AB(300mg/kg)** indicates administration of 300mg/kg aqueous root extract of *Arnebia benthamii* for 14 days.

\*\*= p≤ 0.001 vs control group

\*= p≤0.01 vs control group

## DISCUSSION

The current drug which is used in the treatment of depression includes SSRI's (selective serotonin reuptake inhibitor), TCA (Tricyclic antidepressants) and other synthetic analogues. Along with the pharmacological treatment, the non-pharmacological treatment is also helpful in combating depression, but none offers a complete, harmless cure, it only improves the symptoms of the disease and benefits mood and behavior.

The synthetic antidepressants however have plenty of side effects such as drowsiness, sleepiness, loss of libido and anxiety. Hence, nowadays researchers are focusing on finding new antidepressant leads that are equally effective as synthetic drugs and have minimum side effects therefore plants having antioxidant property are being researched on that can act as antidepressant drugs.

The widely accepted models for assessing antidepressant activity in animal are force swim test and tail suspension test which are used in the present study. Animal are exposed to these models for inducing as well as for assessment of depression. Different groups were given the respective treatment such as normal saline, Imipramine 10 mg/kg. Aqueous root extract of *Arnebia Benthamii* 75, 150 and 300 mg/kg for a period of 14 days. Each treatment group was exposed to behavioral testing and biochemical estimation on the 14<sup>th</sup> day of dosing. There is a rise in free radicals and oxidative stress in depressed rodents exposed to depressive test parameters. Results of the present study demonstrated that these behavioral models were capable of producing depression like state in rodents shown by increase in immobility period in force swim and tail suspension test.

All the groups showed a significant decrease in immobility period as compare to the control group in force swim test and tail suspension test. Aqueous root extract of *Arnebia benthamii* at a dose of 75 mg/kg has less effect in decreasing immobility period as compared to aqueous root extract of *Arnebia benthamii* at 150 and 300 mg/kg thus making 150 and 300 mg/kg more beneficial in improving depression. The effect of aqueous root extract of *Arnebia benthamii* on brain glutathione, malondialdehyde, superoxide dismutase and nitrite level were also assessed to detect the oxidative mechanism behind

the progression of depression in rat brain. Depression is closely associated with increased oxidative stress and the generation of free radicals in the brain region especially the cortex and hippocampal region which are responsible for cognitive functions, mood and behavior. Lipid peroxidation and nitrite (metabolite of nitric oxide) plays a major role in oxidative stress.

It has been reported that the level of malondialdehyde and nitrite are generally higher in individuals with depression and the level of SOD and brain glutathione are generally lower in individual with depression [19, 20, 21].

Treatment with aqueous root extract of *Arnebia benthamii* at a dose of 75, 150 and 300 mg/ kg significantly decreased the level of malondialdehyde and nitrite and it significantly increased the level of SOD and brain glutathione.

Administration of the standard drug Imipramine (10 mg/kg) showed significant improvement in immobility period, increased in SOD and brain glutathione and decrease in brain malondialdehyde levels owing to its antioxidant potential which have been proved by some studies.

## CONCLUSION

In the present investigation the antidepressant activity of aqueous root extract of *Arnebia benthamii* was assessed by force swim test and tail suspension test in rats. Depressive symptoms such as immobility appeared in rats exposed to these behavioral models. Aqueous root extract of *Arnebia benthamii* shows significant improvement in the immobility period by decreasing the immobility period in force swim test and tail suspension test. Higher dose (150 & 300 mg/kg) of aqueous root extract of *Arnebia benthamii* was more effective as compared to the lower dose. Aqueous root extract of *Arnebia benthamii* at higher dose shows significant increased in the SOD as well as in the brain glutathione levels as compared to the control group.

Malondialdehyde levels and nitrite levels were significantly decreased in aqueous root extract of *Arnebia benthamii* treated group especially the higher dose.

Thus, it is evident from the observation that aqueous root extract of *Arnebia benthamii* has proven to be a good antidepressant agent. It can improve the depressive symptoms.

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