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ANTIDEPRESSANT EFFECT OF *AMARYLLIS BELLADONNA* IN VARIOUS ANIMAL MODELS

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ABSTRACT:

Using Forced Swim Test (FST) and Tail Suspension Test (TST), the antidepressant effect was determined. In both animals, prolonged immobility was seen, which was indicative of antidepressant action. The results demonstrate a dose-dependent antidepressant effect of *Amaryllis belladonna* bulb extract in both test models. The extract of *Amaryllis belladonna* possesses potent antidepressant properties.

Keywords: Antidepressant, *Amaryllis belladonna*, FST, TST.

INTRODUCTION:

Depression is marked by sleep, appetite disturbance and cognitive deficits. Depression has impacted millions of people worldwide (1-3). The disease's course is typically repeated(4).

The common name for *Amaryllis belladonna* is belladonna lily. It is traditionally known as March lily in South Africa since it blooms from March to April (5). *Amaryllis belladonna* is a member of the Amaryllidaceae family. It is perennial and propagated by means of bulbs (6). Family Amaryllidaceae plants are of great therapeutic use (7).

In the beginning, remedies were administered as crude pharmaceuticals in the form of infusions, tinctures, decoctions, teas, poultices, powders, and other herbal preparations (8).

Amaryllis belladonna contains phytochemicals such as phenolic acid, terpenoids, and alkaloids. Phenolics acid has antioxidant, cytotoxic, antiulcer, anti-inflammatory, anticancer, and antispasmodic properties, whilst terpenoids and alkaloids (9-12).

Carotenoids, which are responsible for the pigmentation of many plants and fruits, have antioxidant potential. Carotenoids, as essential nutrients, may delay or prevent oxidative damage and provide protection against cancer, cardiovascular disease, eye illnesses, atherosclerosis, and ageing (13-14).

In Vietnamese traditional medicine, Amaryllis belladonna is used as a treatment against tumours (15). These elements include terpenoids, alkaloids, tannins, steroids, carbohydrates, flavonoids, and other bioactive substances that exert a specific physiological effect on the human body (16). Rutin, a flavonoid, is the most major bioactive component identified from plants in the family Amaryllidaceae (17). Rutin has been shown to be effective against inflammations, reactive oxygen species, cancer, and cardiovascular disorders (18).

MATERIAL AND METHODS:

The bulbs of *Amaryllis belladonna* were collected from the local Nursery near railway station (Jammu), district, Jammu, India in December 2018. Herbarium Assistant Mr. Ram Prasad of Department of Botanical & Environmental Sciences (Guru Nanak Dev University, Amritsar carried out authentication of plant. Authentication No: *Ref. No. 1926.*

Preparation of Methanol Extract of *Amaryllis belladonna* (MEAB):

The obtained plant bulbs were dried in the shade. With the assistance of a suitable grinder, the dried plant material was next ground into a coarse powder. Extraction was done with methanol using a soxhlet apparatus. Concentration of the extract was achieved by heidolph rotavator. A dark maroon-colored gelatinous concentration was classified as methanol extract of Amaryllis belladonna (MEAB).

ANIMALS:

Experiments were conducted on albino mice weighing 25-30g of either sex. The animals housed in cages made of polypropylene. The offered bedding material was paddy husk, which was replaced daily. There were six mice each cage. The animals were fed a regular pellet diet and had free access to water. They were housed in a well-ventilated chamber with a 12-hour light and dark cycle. The temperature was maintained at 22-24° Celsius. CPSCEA guidelines were followed. CPCSEA/RE/S/2005/894.

Albino mice (25-30g) were randomly divided into 4 groups of 6 each and received drugs as follows:

G 1: Normal saline (10mg/kg p.o), G 2: Imipramine (30mg/kg p.o), G 3: MEAB-Methanol extract of *Amaryllis belladonna* bulbs (100mg/kg p.o), G 4: MEAB-Methanol extract of *Amaryllis belladonna* bulbs (200mg/kg p.o)

DRUGS AND TREATMENTS:

Imipramine hydrochloride (30mg/kg). Dose of Imipramine was selected based on Literature review (Manan *et al*., 2015).

ANTIDEPRESSANT ACTIVITY TESTS:

Forced Swim Test

Performed as per standard procedure of (Arunachalam G., et al., 2008)

Tail Suspension Test

Performed as per standard procedure of (Arunachalam G., et al., 2008)

Potentiation of Norepinephrine toxicity

Performed as per standard procedure of Alpermann HG et al., 1992.

STATISTICAL ANALYSIS:

All the results are expressed as mean ± SEM followed by one way ANOVA and Dunnett's multiple comparison post hoc tests. The data were statistically analyzed by using graph pad prism version – 6.0 software. The value $p<0.01$ was considered to be statistically significant.

RESULTS:

Phtochemical Screening Test:

Based on phytochemical tests, phytoconstituents present in MEAB were found to be: alkaloids, terpenoids, flavonoids, tannin as depicted in **Table 1.**

Table 1: Results of phytochemical tests of the Methanol extract of*Amaryllis belladonna* bulbs (MEAB).

Extract	Carbohydrates	Tannins	Flavonoid	Saponin	Phenols	Steroids	Alkaloids	Glyco sides
MEAB	-	+	+	-	+	-	+	-

MEAB – Methanol extract of *Amaryllis belladonna*bulbs

(+) – Present

(-) – Absent

ACUTE ORAL TOXICITY STUDY:

The Methanol extract of *Amaryllis belladonna* (MEAB) bulbs did not elicit any toxic symptoms or mortality in mice at oral doses up to 2,000 mg/kg body weight. No toxicity/death was reported at these levels throughout the research. **Table 2** (11)

Table 2: Acute toxicity study on Methanol extract of *Amaryllis belladonna* bulbs

Group	Dose (mg/kg)	No of animals	Dose differences (a)	Mortality (b)
I	50	6		No
II	100	6	50	No
III	500	6	400	No
IV	1000	6	500	No
V	1500	6	500	No
VI	2000	6	500	No

Forced Swim Test:

After 1 hour of vehicle treatment (0.9% NaCl), the duration of immobility in the control group was 184-200sec/6min. The duration of immobility in rats administered Imipramine (30mg/kg/p.o) decreased significantly ($p<0.01$) when compared to the duration of immobility in the control group. The length of immobility decreased significantly ($p<0.01$) as a function of dose in rats treated with MEAB (Group III and Group IV, 100 and 200 mg/kg/p.o.). Group IV (200 mg/kg/p.o.) doses of the test medication. MEAB had a larger effect than the control group, and Group IV had a significantly ($p<0.01$) more potent effect than Group II. After 1 hour of MEAB therapy, animals in Groups III and IV remained immobile for an average of 152 and 128 seconds, respectively, throughout a total test length of 6 minutes.

Table 3:MEAB on FST Induced Duration of Immobility in Mice:

Group	Treatment	Duration of immobility (sec) After 1 hr treatment (Mean ± SEM)		
		Day 1	Day 7	Day 14
I	Normal saline (10ml/kg/p.o.)	190 ± 3.126	188 ± 3.116	185 ± 3.109
II	Imipramine (30 mg/kg/p.o.)	109 ± 2.472 ^{a**}	106 ± 2.462 ^{a**}	102 ± 2.459 ^{a**}
III	MEAB(100 mg/kg/p.o.)	155.2±1.987$^{a**\ b**}$	152 ± 1.980$^{a**\ b**}$	150 ± 1.978 $^{a**\ b**}$
IV	MEAB(200 mg/kg/p.o.)	130 ± 1.569 $^{a**\ b**}$	128 ± 1.565 $^{a**\ b**}$	125 ± 1.560 $^{a**\ b**}$

The mean and SEM of six observations are used to express values. One-way ANOVA and Dunnet's Multiple Comparison Post hoc Test were used to evaluate the data. *p < 0.05, **p < 0.01, ***p < 0.001.[a]Compared with control, [b]Compared with Imipramine.

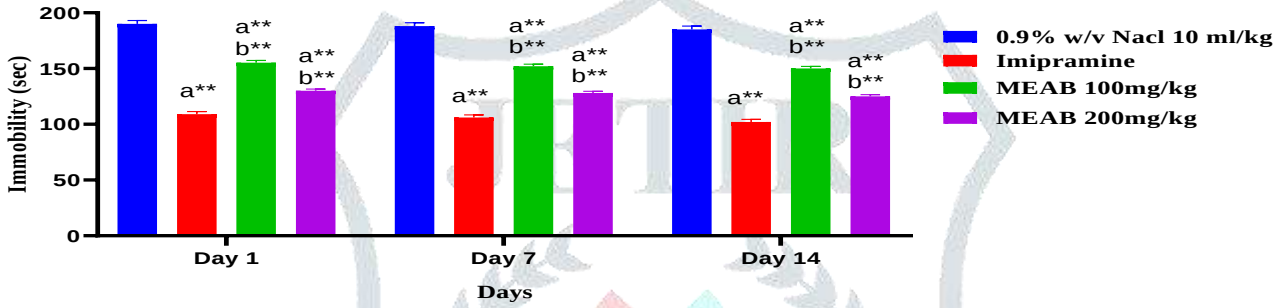

Fig 1:Effect of MEAB on FST Induced Duration of Immobility in Mice.

Tail Suspension Test:

The control group did not move for 195-209 seconds or 6 minutes following an hour of vehicle feeding. When compared to rats in the control group, animals given imipramine (30 mg/kg/p.o.) had significantly shorter periods of immobility ($p<0.01$). In rats given MEAB (Group III and Group IV, 100 and 200 mg/kg/p.o.), the duration of immobility significantly ($p<0.01$) reduced with dose. When compared to the control group, doses of Group IV test drug MEAB (200 mg/kg/p.o.) exhibited a significant ($p<0.01$) effect, but not when compared to the standard ($p<0.05$) (1^{st} and 7^{th} day). Animals in Groups III and IV remained motionless for an average of 151 and 131.6 seconds, respectively, over the course of a 6-minute test after receiving MEAB therapy for one hour.

Table 4:Effect of MEAB on TST Induced Duration of Immobility in Mice:

Groups	Treatment	Duration of immobility (sec) After 1 hr treatment (Mean ± SEM)		
		Day 1	Day 7	Day 14
I	Normal saline(10ml/kg/p.o.)	202.2±3.533	200.3±3.530	199.9±3.529
II	Imipramine (30 mg/kg/p.o.)	120.3±2.579 ^{a**}	119.2±2.576 ^{a**}	117.1±2.573 ^{a**}
III	MEAB(100 mg/kg/p.o.)	150±2.447 $^{a**\ b**}$	148±2.444 $^{a**\ b**}$	145±2.439 $^{a**\ b**}$
IV	MEAB(200 mg/kg/p.o.)	130±2.133 $^{a**\ bns}$	129±2.130 $^{a**\ b*}$	125±2.127$^{a**\ bns}$

The mean and SEM of six observations are used to express values. One-way ANOVA and Dunnet's Multiple Comparison Post hoc Test were used to evaluate the data. *p < 0.05, **p < 0.01, ***p < 0.001.

[a]Compared with control, [b]Compared with Imipramine.

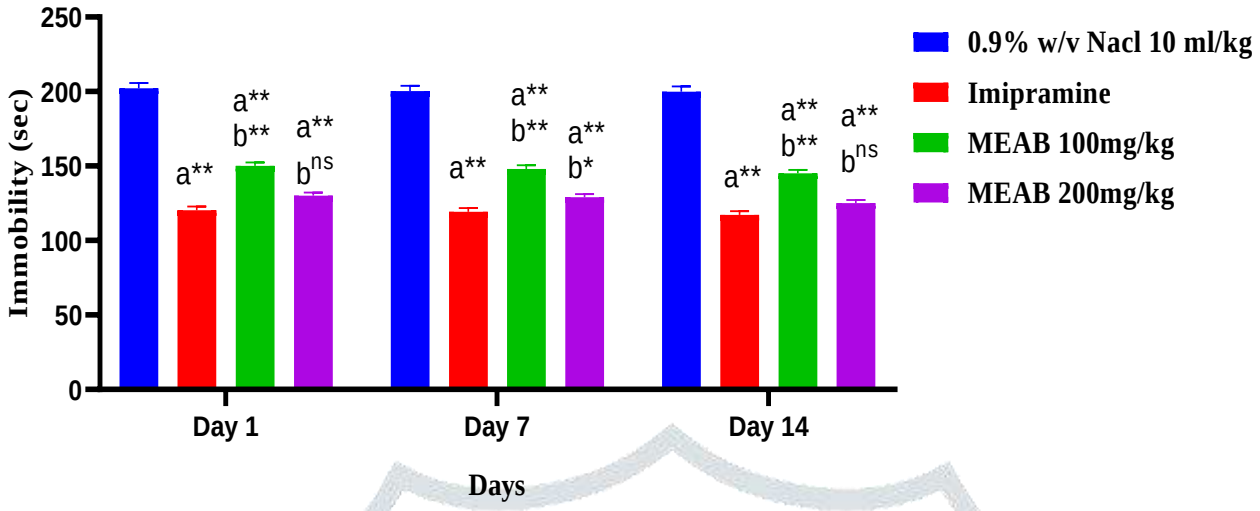

Fig 2: Effect of MEAB on TST Induced Duration of Immobility in Mice.

Potentiation of Nor-epinephrine toxicity:

Mice mortality is not observed in animals provided the vehicle control group (0.9 percent NaCl). Three mice died in animals administered NE (4 mg/kg/i.p.), but animals in Group III who got the usual medicine Imipramine (10 mg/kg/day, twice daily) 30 minutes before NE administration showed a substantial increase in mouse mortality. Three mice died in animals given MEAB (200 mg/kg/p.o. twice day) 30 minutes prior to NE delivery. Imipramine significantly increased NE toxicity in mice, whereas MEAB did not significantly increase NE toxicity in mice **(Table 5).**

Table 5: Effect of MEAB on Norepinephrine potentiation toxicity in mice:

Groups	Treatment	Number of mortalities	Lethality (%)
I (Control)	Normal saline (10 ml/kg/p.o.)	0	0.0
II (NE treated)	NE (4.0 mg/kg/i.p.)	3	50
III (standard+NE)	Imipramine (30 mg/kg/i.p.) + NE (4.0 mg/kg/i.p.)	6	100%
IV (Test + NE)	MEAB (200 mg/kg/p.o.) + NE (4.0 mg/kg/i.p.)	3	50

Discussion:

Depression is a state of poor mood and aversion that can influence an individual's thought, behaviour, feeling, and physical health. The current treatment for depression is centred on the use of synthetic drugs and replacement therapies, both of which have a number of long-term risks and adverse effects. Depression has a significant prevalence in the community and is connected with a great deal of morbidity (19).

The effectiveness of Amaryllis belladonna bulbs against depression has not been demonstrated. Therefore, the objective of this study was to assess the antidepressant properties of Amaryllis belladonna in mice.

In the present work, the following models are utilised: Force swim test, Tail suspension test, and Norepinephrine toxicity potentiation in mice. FST is the most often used animal model of depression. The doses chosen for the current investigation were (100 mg/kg/p.o.) and (200 mg/kg/p.o.) of methanol extract of *Amaryllis Belladonna*.

In preliminary phytochemical analysis of the methanol extract of *Amaryllis belladonna*, flavonoids, phenols, and alkaloids responsible for antidepressant action in animal models were identified.

The acute toxicity investigation revealed that a dose of 2000 mg/kg of MEAB caused neither toxicity nor mortality in mice, indicating its safety. The acute toxicity study was conducted using OECD-423 (acute toxic class method).

In the present investigation, the Force swim test, the Tail suspension test, and the Potentiating of Norepinephrine toxicity in mice were chosen as models purportedly capable of simulating human depression. The forced swim test is generally recognised as an animal model of depression. This strategy experimentally induces depression (20).

Compared to the control group, the doses of MEAB (100 mg/kg/p.o.) and (200 mg/kg/p.o.) significantly reduced the immobility of mice in the Forced swim and Tail suspension tests. Imipramine, administered at a dose of 30 mg/kg/p.o. of body weight, provided 100 percent protection and greatly shortened the time of immobility. The FST shown a high sensitivity to monoamine modifications and is susceptible to monoaminergic interventions. MEAB's antidepressant effects may also be mediated by the flavonoid components' interactions with adrenergic and serotonergic systems. In addition, the effect of MEAB on the enhancement of Norepinephrine's toxicity in mice was examined (21).

Conclusion:

The FST (forced swimming test), the TST (tail suspension test), and the potentiating of nor-epinephrine toxicity were used to observe the antidepressant effect of MEAB. The current study urges more investigation into the identification of the active components in herbal medicines, namely an extract of *Amaryllis belladonna* with antidepressant-like qualities. More research is required to determine its precise mode of action and to pinpoint the component as a strong and effective drug.

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