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EVALUATION OF PRUNUS *AMYGDALUS* SEED EXTRACT FOR ANTIDEPRESSANT ACTIVITY

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Abstract: *Prunus Amygdalus* is highly nutritious plant belonging to family Rosaceae. Antidepressant activity of PA was evaluated using validated animal models of depression. Possible mechanisms involved in antidepressant actions of PA were evaluated using Tail Suspension Test, Haloperidol induce Catalepsy, Locomotor activity models. Dose response study in preliminary Tail Suspension Test revealed the initial antidepressant activity. Significant reduction in duration of catalepsy was shown by PEPA at dose of 400 mg/kg in haloperidol induce catalepsy. TBARs level and glutathione concentration was evaluated to assess the antioxidant action of PEPA against depression induced oxidative stress. PEPA also restored the level of TBARs and glutathione which was altered due to haloperidol administration. Further, reduced locomotor activity due to dexamethasone administration was significantly increased by PEPA treatment. In conclusion, this behavioral and biochemical studies depict the antidepressant action of PEPA in animal models.

Keywords: *Prunus Amygadlus*, Tail suspension test, Haloperidol induce catalepsy, oxidative stress, Locomotor activity.

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INTRODUCTION

Depression is the most prevalent, chronic, recurrent and potentially life threatening psychiatric illness with impact on quality of life of the patients and most significant risk factor for suicide in adolescents, young adults and elderly people[1]. It is one of the leading causes of mortality and morbidity; and according to World Health Organization, by the year 2020, depression will be in the second place as largest contributor to the global burden of diseases [2,3]. The psychopathological state involves a triad of symptoms with low or depressed mood, anhedonia, and low energy or fatigue. Other symptoms, such as sleep and psychomotor disturbances, feelings of guilt, low self-esteem, suicidal tendencies, as well as autonomic and gastrointestinal disturbances, are also often present. Unlike other illnesses or disorders, there is no simple explanation as to what causes depression.[4] In general, depression can be due to a number of factors including stresses which can range from mild to severe, combined with vulnerability or predisposition to depression that can result from biological, genetic or psychological factors[5]. The pathophysiology of depression is hypothesized due to either deficiency in monoamines viz Norepinephrine, 5-HT, Dopamine or Hyperactivation of HPA axis or deficiency in neurotrophic factors. Recent evidence suggests that depression may be associated with neurodegeneration and reduced neurogenesis in the hippocampus. Oxidative stress and nitrosative stress also plays a crucial role on development of depression[6].

Antidepressant drugs are available in market. However, because multiple pathogenic factors are involved in depression, many synthetic antidepressant drugs show slow response and even produce adverse effects such as cardiotoxicity, Hypertensive crisis, sexual dysfunction and sleep disorder in depressant patients.[7] Due to safety concerns and side effects of many antidepressant medications, herbal psychopharmacology research has increased, and herbal remedies are becoming increasingly popular as alternatives to prescribed medications for the treatment of depressive disorder in the last several years. One such plant which claims various medicinal properties is *Prunus Amygdalus* nuts (almond), a popular, nutritious medicinal plant extensively used in Ayurveda. The almond plant is well documented traditionally in ayurveda system of medicine and it is classified in ayurvedic system as 'medhya rasayana' (Nootropics) and are also used in folklore practice [8].

The almond tree is a small deciduous tree which grows to between 4 and 10 meters in height, with a trunk of up to 30 centimeters in diameter. The flowers are pale pink and 3-5 cm in diameter with five petals. Almond nut is 2-3 cm in length, rounded at one end and pointed at the other. They have a thin, cinnamon-brown testa which is easily

removed after soaking in warm water. The oily kernels consist of two large oily planoconvex cotyledons of unequal size and are irregularly folded[9]. Previous studies reported that, almond has good nootropic and hypophagic activity, antidiabetic activity, immunostimulatory activity, aphrodisiac activity, Anti-amnesiac activity etc. Previous studies reported that, almond contains globulin and Amino acids namely trypothan, Cystenin, leucine as a major constituents[10]. The most abundant class of polyphenols in almonds is proanthocyanidins, followed by flavonoids and phenolic acids. Almond flavonoids include flavonols, flavonones, and flavanols, in their monomeric, oligomeric and polymeric forms; these latter also called proanthocyanidins or condensed tannins[11]. Captivating the recent knowledge of almond constituents, the current study was undertaken (i) to investigate the effects of *Prunus Amygdalus* seeds (almond) in validated animal models of depression (ii) to explore the possible underlying mechanism of anti-depressant like activity of almond.

MATERIALS AND METHODS:

Procurement of Animals:

Experiments on animals were approved by the Institutional Animal Ethics Committee of Dr. L. H. Hiranandani college of Pharmacy, opposite to Ulhasnagar station, CHM Campus, Ulhasnagar-03, India (Protocol No. IAEC/PCOL-01/2014). Male Swiss Albino mice (25-30g) were purchased from Bharath serums and vaccines Limited, Plot no. A-371-372, Road NO.27,Wagle Industrial Estate, Thane 400604, Maharashtra, Registration no. 103/99/CPCSEA and maintained in standard laboratory conditions with food (standard pellet chow feed) and filtered water *ad libitum*.

Preparation of Extract [12]

The dried seeds/ nuts of *Prunus Amygdalus* (almond) were collected from local market of Dombivli, Thane district. Almonds were authenticated at the Khalsa College of Arts Commerce And Science, Mumbai (specimen number nk1030702).

Almond powder was first defatted with hexane. Defatted almond residue was then extracted with aqueous acedified acetone. (ratio:- 70:29.5:0.5 of acetone, distilled water, and acetic acid). Mixture was vortexed for 30−40 s, and sonicated in an ultrasonic cleaner for 10 min at 37 °C. Sample was then agitated on orbital shaker for 24 hrs at room temperature. Proanthocyanidin rich extract was filtered using Buchner funnel equipped with an appropriately sized Whatman no. 1 filter and acetone was removed on a rotary evaporator equipped with a vacuum pump, at 40°C.

Purification of extract

Aqueous extract of Proanthocyanidin was extracted with chloroform. Residual organic solvent was removed on a rotary evaporator equipped with a vacuum pump, at 40°C. Crude extract was freeze dried and kept in desiccators filled with silica desiccant.

Phytochemical Testing of Extract [13,14]

The freshly prepared crude extracts were qualitatively tested for the presence of chemical constituents such as flavonoids, proanthocyanidins, alkaloids, tannins and phenolic acids. These were identified by characteristic colour changes using standard procedures.

TLC Analysis of Extract [15]

TLC profile of PEPA was developed to confirm the presence of proanthocyanidins in extract using catechin as a standard. The solvent system used was Toluene(3):Acetone(3):Acetic acid.

Acute toxicity study [16]

Acute toxicity study of test drug was performed on Swiss albino mice (Male) weighing around 25-30 g according to OECD guidelines 423. Animals were fasted overnight and then test substance (PEPA) was provided with single oral dose of 2000mg/kg. Mice were observed individually after dosing at least once during the first 30 minutes, periodically during the first 24 hours, with special attention given during the first 4 hours and daily thereafter upto 14 days.

Pharmacological Activity

In Vivo Antidepressant activity:

1. **Tail Suspension Test**:

Overnight fasted Swiss albino mice were randomly divided into three groups of six animals each.

The Control group received normal saline one hour before the test. Single administration of Standard (Imipramine) and test substance (PEPA) were given one hour prior to test. After one hour of administration, mice were being individually suspended 50 cm above the surface of table with an adhesive tape placed 1 cm away from the tip of the tail. Immobility period was measured for the last five minutes during six minutes using a stop watch. Mice were being considered immobile only when they hung passively and were completely motionless.[17]

2. **Haloperidol induce catalepsy** [18]

Overnight fasted Swiss albino mice were randomly divided into three groups of six animals each.

Mice were divided into four groups of six animals each. Group I served as normal control that received normal saline. Group II served as disease control that received haloperidol. Group III served as standard group that received flouxetine, and haloperidol. Group IV served as drug treated group that received PEPA and haloperidol. All these doses were given to mice for seven days. The duration of catalepsy was measured at 30, 60,90,120,150,180 min. Catalepsy was assessed by means of standard bar test on every 3rd, 5th and 7th day of the drug treatment. The catalepsy was measured in time for which the mouse maintained an imposed posture with both the front limbs extended and resting on a 4 cm high wooden bar (1.0cm diameter). The end point of the catalepsy was considered to occur when both the front paws were removed from the bar or if the animal moved its head in an exploratory manner. A cut-off time of 200 seconds was applied. The effects of the test drug PEPA and the standard drug flouxetine were assessed after their repeated dose administration in mice for seven days, 30 minutes prior to the administration of haloperidol. The mice were sacrificed on the 7^{th} day and the TBARS, glutathione were estimated.

Biochemical parameters:

Preparation of brain homogenate

On 7th day of dosing of all groups, for the biochemical analysis, animals were scarified by anesthetized with by using CO_2 chamber immediately after behavioral assessment. The brains were removed and rinsed with 0.9%Nacl solution and weighed. Tissue was homogenized with phosphate buffer solution (ph-8) for 1 minute and then centrifuged the homogenized mixture for 10 min at 2000 rpm. An aliquot supernatant phase was collected and stored at 2-8^{o} C.

- **Estimation of lipid peroxidation:**

The supernatant of the tissue homogenate was treated with 10 % TCA reagent in 1:1 ration and kept the solution in ice bath for 15 minutes. Solution was then centrifuged for 5 minutes at 2000 rpm. 1 ml of supernatant was taken out and mixed with freshly prepared 2 ml of 0.67% TBA solution. The mixture was kept in boiling water bath for 15 minutes. After cooling, the tubes were centrifuged for 10 minutes and the supernatant taken for measurement. The developed color was read at 532 nm using a UV spectrophotometer (Shimadzu UV-1700, UV-VIS double-beam Spectrophotometer) against a reagent blank. The TBARS concentration was expressed as nM/mg of tissue.

- **Estimation of reduced gluthathione**

The supernatant of brain homogenate was mixed with trichloroacetic acid (10% w/v) in 1:1 ratio.The tubes were centrifuged at 10000 rpm for 10 min. at 4^{o}C.The supernatant obtained (0.8 ml) was mixed with phosphate buffer and 0.01 M DTNB reagent. Absorbance was noted

spectrophotometrically (Shimadzu UV-1700, UV-VIS double beam Spectrophotometer) at 412 nm. Concentration of reduced glutathione was measured and expressed as nM/mg of tissue.

3. Locomotor activity by actophotometer [19]

Overnight fasted animals were randomly divided into four groups of six animals each. Dexamethasone was administered to all groups of mice except saline treated group. Acute stress (Forced swim) was given to mice 30 min after dexamethasone administration. Standard group and test substance group received respective drugs 180 min. after dexamethasone administration. Control group received normal saline 30 min before the test. Locomotor activity was evaluated 3.5 hr after dexamethasone administration.

STATISTICAL ANALYSIS:

The results of Antidepressant activity are expresses as mean + SEM from 6 animals in each group. Results were statistically analyzed using one-way ANNOVA followed by Dunnett's multiple comparison test; $P < 0.05$ was considered significant. Graph Pad Prism 5.03 was the software used for statistical analysis.

RESULTS:

Pharmacognostical Activity:

The preliminary phytochemical screening of PEPA revealed the presence of Flavonoids, Tannins and Phenolic compounds and amino acids.

TLC Profile of PEPA

Rf value for PEPA was found to be 0.52 & Rf Value for standard catechin was found to be 0.54. Which was similar to each other thus suggested presence of proanthocyanidin in PEPA.

Acute Toxicity Study:

In the acute toxicity study no deaths were observed. PEPA did not show any toxic or deleterious effects up to 2000 mg/ kg oral dose. The animals showed no symptoms associated with toxicity.

In-Vivo Study

1. **Tail Suspension Test**

Groups	Treatments	Duration of Immobility (Sec)
Control	Normal Saline (5 ml/kg,p.o.)	151.5 ±0.76
Standard	Imipramine (20 mg/kg, p.o.)	73.33±1.054*

Test drug	PEPA (400 mg/kg, p.o.)	85.00±1.00*

Table No. 1: Effect of PEPA on duration of immobility.

All values are expressed in mean ± SEM for 6 animals in each group (n=6). Significance was determined by One-way ANOVA followed by Dunnett's multiple comparison tests.

*: p<0.001 when compared with Vehicle control.

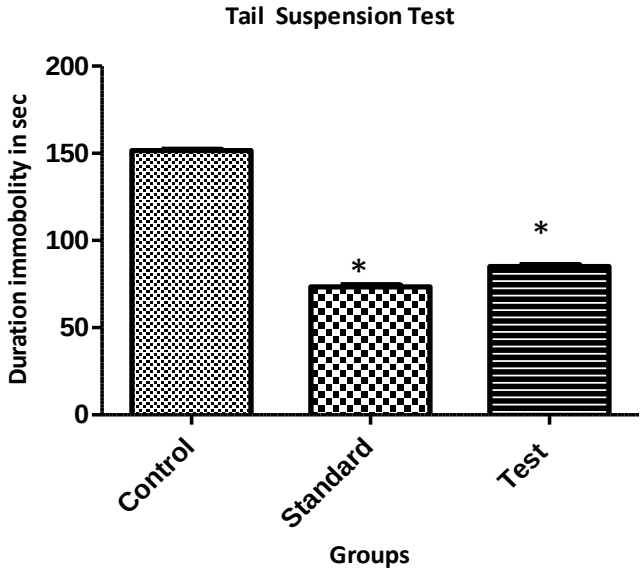

Figure No. 3: Effect of PEPA on duration of immobility.

2. Haloperidol induce catalepsy

Groups	Duration of catalepsy (Sec)					
	30 min	60 min	90 min	120 min	150 min	180 min
Control **Normal saline** **(5ml/kg, p.o.)**	0.6667 ± 0.2108	1±0	1±0	1±0	1±0	1±0

Disease control Haloperidol (1 mg/kg, i.p.)	158.5± 0.7638	161.5± 0.4282	163.8±0.6009	166.7±0.667	170.3±0.667	172.3±0.4944
Standard Fluoxetine (5 mg/kg, i.p.)	74.67± 0.7149*	77± 0.9309*	80.17±0.7032*	82.83±0.7923*	84.67±0.9888*	86±0.8944*
Test drug PEPA (400 mg/kg,p.o.)	98.83± 1.222*	101.5± 1.522*	105.7±1.453*	108.8±1.447*	111.8±1.249*	114.2±.249*

Table No.2: Effect of PEPA on catalepsy on 3rd day at different time interval.

All values are expressed in mean ± SEM for 6 animals in each group (n=6). Significance was determined by One-way ANOVA followed by Dunnett's multiple comparison tests.

*: $p<0.001$ when compared with disease control.

Groups	Treatment	Duration of catalepsy (sec)					
		30 min	60 min	90 min	120 min	150 min	180 min
Control Normal saline (5ml/kg, p.o.)	Normal saline (5ml/kg, p.o.)	1±0.0	1±0.0	1.333± 1.667	1±0.0	1.333± 0.2108	1.833± 0.1667
Disease control Haloperidol (1 mg/kg, i.p.)	Haloperidol (1 mg/kg, i.p.)	167.5± 0.7638	170.3± 0.6146	172.8± 0.5426	174.7± 0.5578	177.3± 0.7601	179.8± 0.6009

Standard Fluoxetine (5 mg/kg, i.p.)	Fluoxetine (5 mg/kg, i.p.)	87± 1.000*	89.5± 0.9574*	92.5± 0.8062*	95.83± 0.9458*	98.33± 0.8433*	99.50± 0.8851*
Test drug PEPA (400 mg/kg,p.o.)	PEPA (400 mg/kg,p.o.)	105.3± 0.7149*	107.7± 0.6667*	109.3± 2.028*	113.2± 0.7032*	117.2± 0.65408	119.8± 0.6009*

Table No.3: Effect of PEPA on catalepsy on 5th day at different time interval

Values are expressed in mean ± SEM for 6 animals in each group (n=6). Significance was determined by One-way ANOVA followed by Dunnett's multiple comparison tests.

*: p<0.001 when compared with disease control.

Groups	Duration of catalepsy (Sec)					
	30 min	**60 min**	**90 min**	**120min**	**150 min**	**180 min**
Control Normal saline (5ml/kg, p.o.)	1.000±0.0	1.000±0.0	1.333± 0.2108	1.667± 0.2108	2.000±0.0	1.833± 0.1667
Disease control Haloperidol (1 mg/kg, i.p.)	175.0± 0.5774	178.2± 0.6009	181.3± 0.3333	184.3± 0.4944	187.5± 0.4282	190.5± 0.4282
Standard Fluoxetine (5 mg/kg, i.p.)	93.17± 0.6009*	95.50± 0.6708*	98.17± 0.8333*	101.3± 0.8028*	103.7± 0.6667*	105.8± 0.79238*
Test drug PEPA (400 mg/kg,p.o.)	115.0± 0.57748	117.3± 0.7601*	120.0± 0.8165*	122.2± 0.6009*	123.7± 0.4216*	126.2± 0.60098

Table No. 4: Effect of PEPA on catalepsy on 7th day at different time interval

Values are expressed in mean ± SEM for 6 animals in each group (n=6). Significance was determined by One-way ANOVA followed by Dunnett's multiple comparison tests.

*: p<0.001 when compared with disease control.

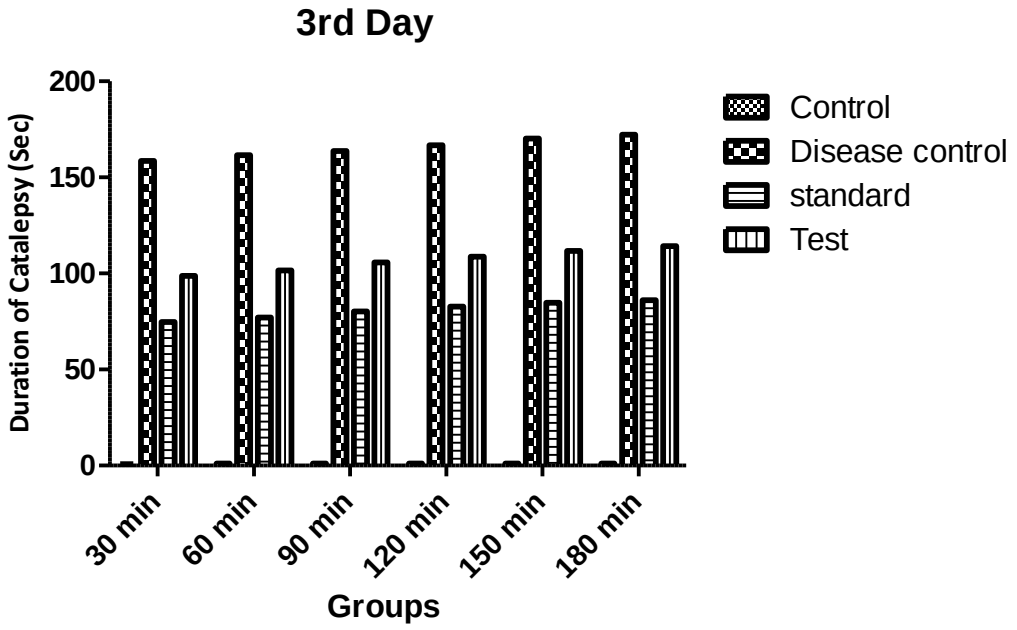

Figure No. 4: Effect of PEPA on catalepsy on 3rd day at different time interval

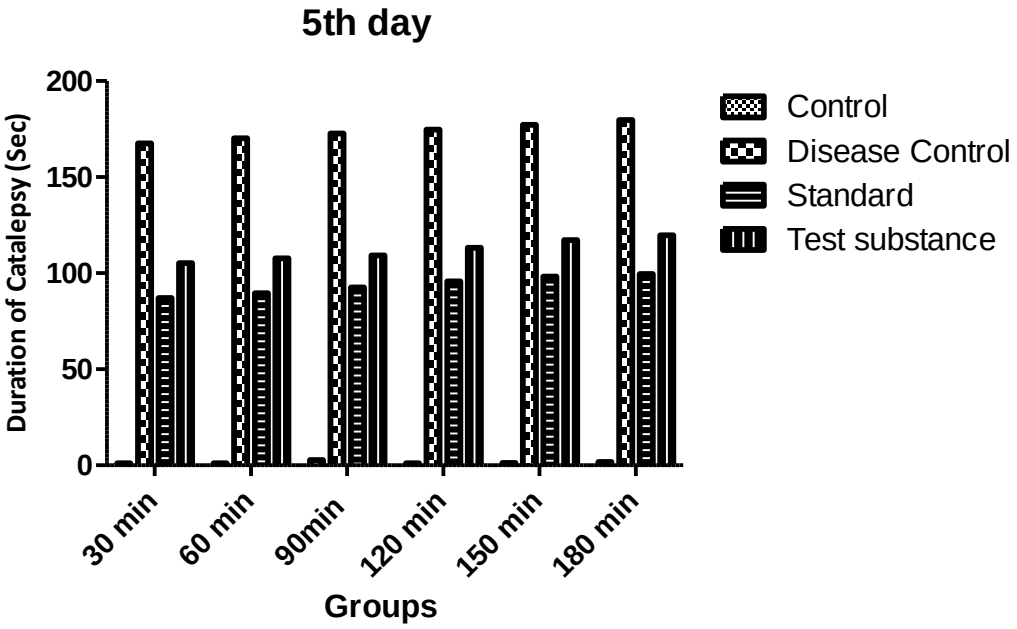

Figure No.5: Effect of PEPA on catalepsy on 5th day at different time interval

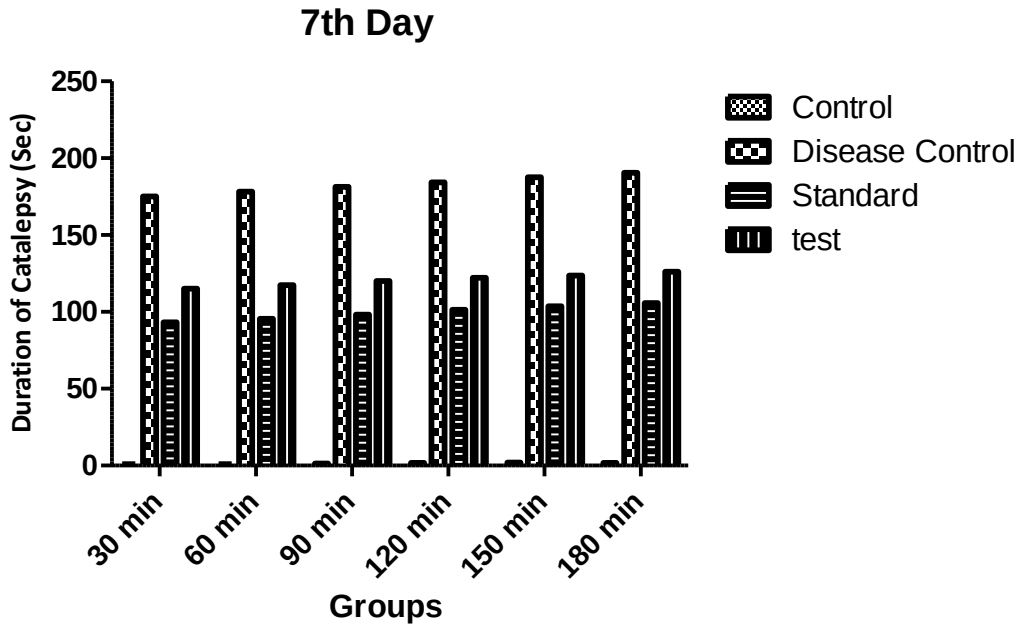

Figure No.6: Effect of PEPA on catalepsy on 7th day at different time interval

Biochemical Parameter

1. Effect of PEPA on Lipid Peroxidation

Groups	Treatments	Concentration of MDA in nM/mg of Tissue
Control	Normal saline (5ml/kg, p.o.)	5.065 ± 0.036
Disease Control	Haloperidol (1 mg/kg, i.p.)	12.39±0.4024
Standard	Fluoxetine (5 mg/kg, i.p.)	7.37 ± 0.0552*
Test drug	PEPA (400 mg/kg,p.o.)	8.163± 0.0215*

Table No.5: Effect of PEPA on lipid peroxidation

All values are expressed in mean ± SEM for 6 animals in each group (n=6). Significance was determined by One-way ANOVA followed by Dunnett's multiple comparison tests.

*: $p<0.001$ when compared with disease control.

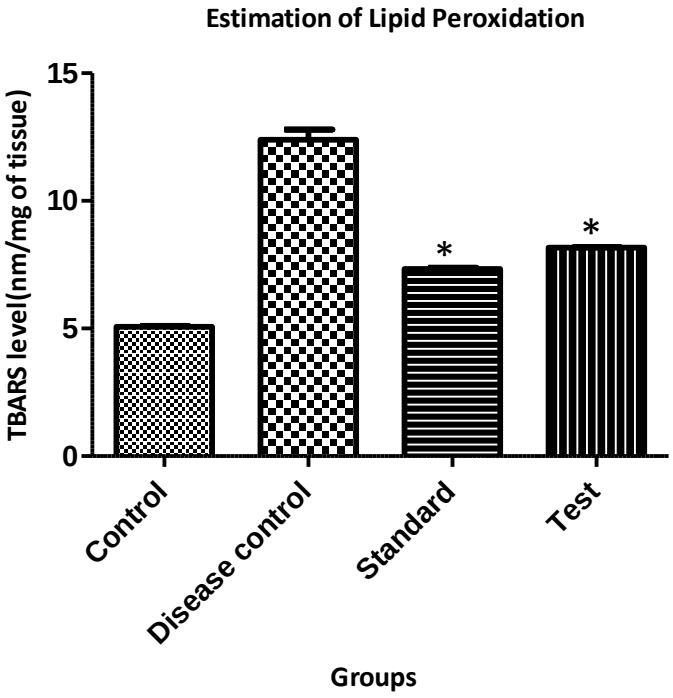

Figure No. 7: Effect of PEPA on lipid peroxidation

2. Reduced glutathione estimation

Groups	Treatments	Concentration of GSH in nM/mg of Tissue
Control	Normal saline (5ml/kg, p.o.)	26.41± 0.1602
Disease Control	Haloperidol (1 mg/kg, i.p.)	7.74± 0.0763
Standard	Fluoxetine (5 mg/kg, i.p.)	18.93 ± 0.1198*
Test drug	PEPA (400 mg/kg,p.o.)	15.89± 0.1543*

Table No.6 Effect of PEPA on glutathione concentration

All values are expressed in mean ± SEM for 6 animals in each group (n=6). Significance was determined by One-way ANOVA followed by Dunnett's multiple comparison tests.

*: p<0.001 when compared with disease control.

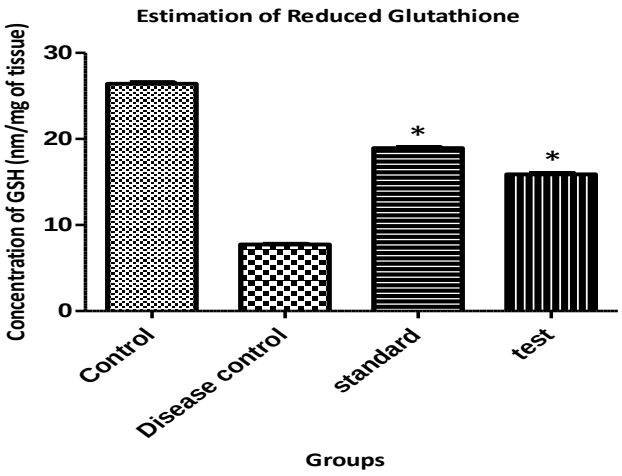

Figure No. 8: Effect of PEPA on glutathione concentration

3. Locomotor activity

Groups	Treatment	Locomotor activity counts/ 10 min.
Control	Normal Saline (5 ml/kg,p.o.)	400 ± 1.118
Disease Control	Dexamethasone (8 mg/kg,s.c)	302 ± 2.671
Standard	Fluoxetine (5 mg/kg, i.p.)	377 ± 1.461*
Test drug	PEPA (400 mg/kg, p.o.)	355 ± 1.302*

Table No. 7: Effect of PEPA on locomotor activity.

All values are expressed in mean ± SEM for 6 animals in each group (n=6). Significance was determined by One-way ANOVA followed by Dunnett's multiple comparison tests.

*: p<0.001 when compared with disease control.

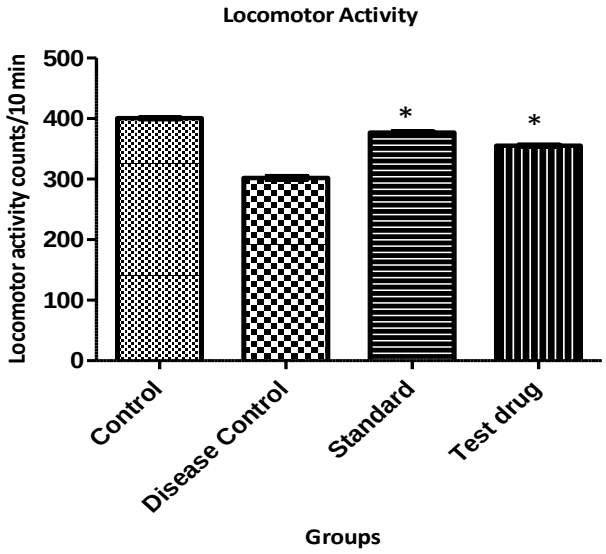

Figure No. 9: Effect of PEPA on locomotor activity.

DISCUSSION:

The purpose of the present study was to evaluate the antidepressant effect of proanthocyanidin rich extract of *Prunus amygdalus* seeds by using animal models and this study revealed that nuts of prunus amygdalus (almond) may be helpful for treating depressive disorder. In phytochemical study, the presence of flavonoids and tannins were observed. Recently several studies have shown the antidepressant activity of flavonoids. The TLC profile revealed the presence of proanthocyanidin in extract. This proanthocyanidin has been proved to be a MAO inhibitor. MAO inhibitors act by inhibiting monoamine oxidase, thus preventing the breakdown of monoamine neurotransmitters and thereby increasing their availability[22]. Therefore one of the antidepressant mechanisms of PEPA is thought to be involving a proanthocyanidin which exerts an MAO inhibitory activity.

Tail suspension test is reliable and rapid screening method for antidepressants, including those involving serotonergic system. This animal model for testing antidepressant activity is based on the principle that suspending mice upside down leads to a characteristic behavior of immobility after initial momentary struggle. This behavior reflects a state of despair, which can be reduced by several agents that are therapeutically effective in human depression. In Tail Suspension Test the immobility parameter was evaluated 30 minutes after the administration of all the drugs. The changes in the duration of immobility are summarized as shown in the table no.1 The PEPA at the dose of 400 mg/kg significantly reduced the immobility duration when compared with vehicle treated group. Hence, in other words the effect of 400 mg/kg can be considered as an antidepressant dose of formulation. Imipramine (20 mg/kg), the standard anti-depressant also

produced the significant decrease in immobility time. Immobility behavior is sensitive to serotonergic agents, such as the SSRI's agents. Based on these findings it can be suggested that the PEPA which is able to reduce immobility duration in mice can exert its effect through a mechanism similar to that of SSR's via serotonin system. More over Imipramine belongs to the class of tricyclic antidepressant drugs was used as standard. These drugs inhibit the reuptake of nor-epinephrine and 5-HT into their respective neurons. Hence PEPA may also mediate its activity through the same mechanism as that of Imipramine.

Behavioral parameter i.e catalepsy and biochemical parameters (TBARs and reduced gluthathione) were evaluated in Haloperidol induce catalepsy model. Effect of PEPA on catalepsy is shown in table no.2,3 and 4. The cataleptic behavior (inability to correct abnormal posture) of haloperidol (1 mg/kg, i.p.) treated animals was found to increase significantly on 3^{rd}, 5^{th}, and also on 7^{th} day of treatment when compared to control group animals. PEPA (400 mg/kg, p.o.) given 30 minute prior to haloperidol, significantly reduced ($p<0.001$) the cataleptic duration when compared to haloperidol treated group on 3^{rd},$5^{th}$and also on 7^{th} day. Fluoxetine (5 mg/kg,i.p.), the standard antidepressant also produced a significant decrease in duration of catalepsy. Various studies reported that catalepsy occurs due to dopamine blockade. Haloperidol induced catalepsy is also associated with an increase in oxidative stress in the brain It has been reported that Fluoxetine attenuates the cataleptic effect which was produced due to haloperidol. This proves that serotonergic pathway modulates the cataleptic behavior. Effect of PEPA on Haloperidol induced lipid peroxidation is shown in table no. 5. Haloperidol treatment to mice for 7 days induced lipid peroxidation as indicated by a significant increase in brain MDA levels when compared with vehicle saline treated animals. Pretreatment with PEPA (400 mg/kg) and Fluoxetine (5 mg/kg) showed significant reduction ($P<0.001$) in extent of lipid peroxidation when compared with haloperidol treated animals. Effect of PEPA on GSh level shown in table no.6. Statistical analysis of brain GSH levels showed a significant difference ($P<0.001$) between the PEPA treated and haloperidol treated mice. Administration of Fluoxetine (5mg/kg, i.p) and PEPA (400mg/kg, p.o) to haloperidol treated animlas significantly increased GSH levels as compared to haloperidol treated group. On the basis of these findings it can be presumed that PEPA shows antidepressant activity by modulating dopaminergic/sertonergic pathway or by reducing oxidative stress which was induced by haloperidol.

Effect of PEPA on locomotor activity was evaluated using Dexamethasone as a depressogenic drug. In the present study,(Table no. 7) the animals that were treated with PEPA at dose of 400 mg/kg showed significant ($P<0.001$) increase in locomotor activity counts when compared with dexamethasone treated animals. This showed that PEPA has reversed the actions of dexamethasone. Dose dependent depressogenic effect of dexamethasone has been shown by

several researchers. Moreover, it has been indicated that exposure to glucocorticoid includind dexamethasone, reduces the neurogenesis in all age groups of animals[23]. Hyanes et al, demonstrated that even a single dose of dexamethasone induces damage to the neurons located in the brain regions associated with mood disorder such as depression. This evidence provides the possibility that PEPA may exert antidepressant actions by promoting neurogenesis which was thought to be decreased by dexamethasone administration. Dexamethasone also exerts numerous effects on dopaminergic pathway hence it can be speculated that antidepressant effect of PEPA might be related to modulation of dopaminergic pathways.

CONCLUSION:

The finding of the present investigation suggests the antidepressant activity of Prunus amygdalus in Tail Suspension Test, Haloperidol induce catalepsy and locomotor activity models of depression. The action of PEPA as an antidepressant may be due to either single or combination of mechanism such as inhibition of reuptake of monoamines, or by reducing oxidative stress or by promoting neurogenesis or due to presence of proanthocyanidin in extract.

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