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EAT PINEAPPLE A DAY TO KEEP DEPRESSION AT BAY

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ABSTRACT
Pineapple, a juicy and tasty fruit, belonging to family Bromeliaceae is scientifically known as *Ananas cosmosus*. The Pineapples are traditionally used as a blood purifier, to aid digestion, for gastro-intestinal disorders, diseases of the larynx and pharynx, as a mild antiseptic and to treat diabetes. There are no reports in literature pertaining to CNS actions of *Ananas cosmosus* fruit. In the light of above, the present study was undertaken to test the antidepressant potential of *Ananas cosmosus* fruit juice. *Ananas cosmosus* juice (ACJ) was administered at various concentrations ranging from 5% to 20% v/v to Swiss albino mice for 15 days and wistar rats for 8 successive days. The antidepressant activity was measured using forced swim test (FST), tail suspension test (TST) and reserpine induced hypothermia. The efficacy of Pineapple juice was compared with standard anti-depressant agents *viz*: fluoxetine (20 mg/kg) and imipramine (15 mg/kg). The results showed that Pineapple juice significantly decreased immobility time in both FST and TST models. It also reversed the hypothermia induced by reserpine. The efficacy of Pineapple juice was found to be comparable to fluoxetine and imipramine. Prazosin, sulpiride, baclofen and p-CPA antagonized the antidepressant effect of Pineapple juice in tail suspension test. Furthermore, *Ananas cosmosus* juice inhibited the monoamine oxidase MAO-A and MAO-B activity and reduced significantly malondialdehyde (MDA) levels. These findings reveal the anti-depressant potential of Pineapple.

KEY WORDS: *Ananas cosmosus,* Anti-depressant, Despair, Immobility

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INTRODUCTION

Mental depression is a complex disorder of unknown etiology, which is manifested by low mood, anhedonia, low energy levels, pessimism, guilty feeling and suicidal tendencies. It may range from a very mild condition, bordering on normality, to severe depression–sometimes called ''psychotic depression'' accompanied by hallucinations and delusions. Patients with major depression have symptoms that reflect changes in brain monoamine neurotransmitters, specifically norepinephrine, serotonin and dopamine.[1] However, most of the marketed anti-depressant drugs exhibit serious side-effects. Therefore, the use of alternative medicines is increasing worldwide. Various herbal drugs (e.g. St. John's wort) have shown promising results in treating experimental as well as clinical depression and many of these herbal drugs appear to be quite safe.[2]

Ananas cosmosus Linn (Bromeliaceae) is commonly known as Pineapple. Pineapple contains several pharmacologically active phytochemicals such as ananasate, beta-sitosterol, chlorogenic acid, rutin, naringenin[3], bromelain[4], glycosides, flavonoids[5] and neurotransmitters such as serotonin[6], dopamine, adrenaline and noradrenaline.[7] *Ananas cosmosus* is reported to possess several medicinal properties such as anti-diabetic[8], anti-tumor[9], anti-oxidant[10], anti-inflammatory[4], immuno-modulatory[11], hepato-protective[12], platelet aggregation activity[13] and anthelmintic activity.[14] However, there is no scientific evidence for the therapeutic potential of Pineapple in neuropsychiatric disorders. Since serotonin and noradrenaline levels fall considerably in depression, we were interested to investigate the usefulness of Pineapple in depression, since Pineapple is reported to contain high amounts of serotonin and fair amounts of adrenaline and noradrenaline.

MATERIALS AND METHODS

Objectives

The present study was undertaken to explore the anti-depressant potential of *Ananas cosmosus* juice (ACJ) using forced swim test, tail suspension test and reserpine induced hypothermia model. An attempt has also been made to determine the underlying mechanism of action of ACJ by co-administration of agents modulating noradrenaline and serotonin.

Plant material

The fresh Pineapples (*Ananas cosmosus*) were purchased from local market of Hisar and got authenticated from Raw Materials Herbarium and Museum, National Institute of Science Communication and Information Resources, New Delhi. Different concentrations of Ananas cosmosus juice (5, 10, 20%, v/v, p.o.) were administered daily for a duration of 8 days to rats and 15 days to mice.

Animals

A total of 204 Swiss mice divided in 34 groups & 36 Wistar rats divided in 06 groups were employed in the present study. Each group comprised of a minimum of 6 animals. Adult (3-4 months old) mice weighing around 20-25 g and adult rats weighing around 80-100 g were procured from the Disease-Free Small Animal House of C.C.S. Haryana Agricultural University, Hisar. The experimental protocol was approved by the Institutional Animals Ethics Committee and the care of laboratory animals was taken as per the guidelines of CPCSEA, Ministry of Forests and Environment, Government of India (Registration number 0436).

Drug protocol

Mice belonging to group I were employed for preliminary studies carried out to determine the effective concentrations of *Ananas cosmosus* Juice (ACJ). Mice belonging to groups II to XV were subjected to Tail suspension test (TST). Mice belonging to groups XVI to XXI were subjected to Forced Swim Test (FST). Mice belonging to groups XXII to XXX were used for biochemical estimations. Mice belonging to groups XXXI to XXXIV were exposed to Photoactometer for assessing the locomotor activity. Rats belonging to groups XXXV to XL were employed in Reserpine induced hypothermia model.

Ananas cosmosus Juice (ACJ) was administered in different concentrations (1% to 40 % v/v, p.o.) in preliminary studies to determine sub maximal effective concentrations of ACJ for further studies. Distilled water (vehicle, p.o.), Fluoxetine (20 mg/kg, p.o.), Imipramine (15 mg/kg, p.o.), Phenelzine (20 mg/kg, p.o.), Prazosin (62.5 mg/kg, i.p.), p-CPA (100 mg/kg, i.p.), Baclofen (10 mg/kg, i.p) , Sulpiride (50 mg/kg, i.p) and ACJ in different concentrations (5%, 10% and 20%, v/v), were administered orally for 15 successive days. At 60 min after administration of the drugs/ distilled water/ ACJ on 15th day, duration of immobility was recorded in mice in TST, FST and biochemical studies. Effect on locomotor activity of mice was studied using a photoactometer. Similarly, distilled water (vehicle), Fluoxetine (20 mg/kg), Imipramine (15 mg/kg) and ACJ in different concentrations (5%, 10% and 20%, v/v), were administered orally for 8 successive days to rats for hypothermia studies. The rectal temperature was recorded on days, 0, 6, 7 and 8. Rectal temperature was measured immediately before and 18h after administration of reserpine, in reserpine induced hypothermia model.

EXPERIMENTAL DESIGN

Tail Suspension Test

Tail suspension test (TST) is a commonly employed model to evaluate new anti-depressant medicines.[15] Immobility reflects a state of helplessness, which can be reversed by drugs such as imipramine and fluoxetine, effective clinically in human depression. The index of depression in this experimental model is taken as the immobility duration over a specific period of time. The increase in immobility period indicates the depressed state of mind. Whereas, the reduction in immobility period exhibits a depression-free state of mind. Mice were suspended on the edge of a table 50 cm above the floor by the adhesive tape placed approximately one cm from the tip of the tail. Immobility time was recorded during a 6 min period.[16] Animal was considered to be immobile, when it did not show any movement of body and hanged passively.

Forced Swim Test

Forced swim test (FST) was proposed as another model to test antidepressant activity.[17] In this model, mice are forced to swim in a restricted space from which they cannot escape. This induces a state of behavioral despair in mice as reflected by increased immobility period, which is similar to human depression.[18] The index of depression in this experimental model is taken as the immobility duration over a specific period of time. Mice were forced to swim individually in a glass jar (25 cm x 12 cm x 25 cm) containing 15 cm deep fresh water and maintained at 25^{0}C (±3^{0}C). After an initial 2 min period of vigorous activity, each animal assumed a typical immobile posture. A mouse was considered to be immobile, when it remained floating in the water without struggling, making only minimum movements of its limbs necessary to keep its head above water. The total duration of immobility was recorded during the next 4 min of total 6 min test. The changes in immobility duration were studied after administering drugs in separate groups of animals. Each animal was used only once.

Reserpine Induced Hypothermia Model

Reserpine induced hypothermia model is commonly employed to evaluate new anti-depressant medicines.[3] Depletion of biogenic amines (noradrenaline, 5-hydroxytryptamine, dopamine) in the brain induces hypothermia in rodents. The decrease in the body temperature indicates hypothermia, the state of depression. Whereas, the increase in the body temperature indicates hyperthermia, a depression- free state of mind.[19] On 6th day, animals were injected with reserpine (2 mg/kg, s.c.), 60 minutes after feeding orally ACJ in different concentrations (5%, 10% and 20%, v/v) to separate groups of rats. Simultaneously, distilled water/ imipramine/ fluoxetine were administered for 8 days to parallel groups of rats. The rectal temperature was determined by insertion of rectal thermometer to a constant depth of 2 cm after eighteen hours of reserpine injection.

Statistical analysis

All the results were expressed as mean ± Standard Error (SEM). Data were analyzed by one-way ANOVA followed by Dunnett's t-test.

RESULTS AND DISCUSSION

In Tail Suspension Test, immobility reflects a state of helplessness, which can be reversed by drugs such as imipramine and fluoxetine, effective clinically in human depression. The index of depression in this experimental model is taken as the immobility duration over a specific period of time. The increase in immobility period indicates the depressed state of mind. Whereas,the reduction in immobility period exhibits a depression-free state of mind. ACJ in different concentrations (5, 10 and 20%, v/v, p.o.), when administered for 15 successive days to mice, decreased the duration of immobility significantly. The antidepressant efficacy of ACJ was found to be comparable to that of fluoxetine (5-HT reuptake inhibitor) and imipramine (Tricyclic anti-depressant) (**Fig. 1**).

In Tail Suspension Test, ACJ in different concentrations (5, 10 and 20%, v/v, p.o.) *per se* diminished the duration of immobility significantly. On the other hand, prazosin (62.5 mg/kg i.p.), p-CPA (100 mg/kg, i.p.), baclofen (10 mg/kg, i.p) and sulpiride (50 mg/kg, i.p) *per se* increased significantly the immobility period of mice. Prazosin/p-CPA /baclofen and sulpiride, when administered on day 15, 45 min after the oral feeding of ACJ 10% v/v reversed the diminished immobility time observed with ACJ (**Fig.7**).

In Forced Swim Test, mice are forced to swim in a restricted space from which they cannot escape. This induces a state of behavioral despair in mice as reflected by increased immobility, which is similar to human depression. The index of depression in this experimental model is taken as the immobility duration over a specific period of time. ACJ in different concentrations (5, 10 and 20%, v/v, p.o.), when administered for 15 successive days to mice, decreased the duration of immobility significantly. The antidepressant efficacy of ACJ was found to be comparable to that of fluoxetine (5-HT reuptake inhibitor) and imipramine (Tricyclic anti-depressant) (**Fig. 2**).

Hypothermia was induced with the help of reserpine (2 mg/kg, s.c.) in rats. ACJ in different concentrations (10 and 20%, v/v, p.o.), when administered for 8 successive days to rats, reversed the hypothermia induced by reserpine. This effect of ACJ was found to be similar to that of fluoxetine (5-HT reuptake inhibitor) and imipramine (Tricyclic anti-depressant) (**Fig. 3**).

Ananas cosmosus Juice (10% v/v) administered to mice for 15 successive days, significantly reduced the brain MAO-A (55.74 ± 4.23 nmol/mg protein) and MAO-B (57.7 ± 4.2 nmol/mg protein) activity as compared to the control group. Furthermore, ACJ (Fig.4, 5 & 6) produced a significant decrease in brain MDA levels (77.7 ± 4.2 nmol/mg tissue) as compared to the control group of mice (105.5 ± 8.8 nmol/ mg tissue).

Anti-depressant potential of Ananas cosmosus juice (ACJ) was tested in mice employing three standard experimental models viz: Forced swim test (FST), Tail suspension test (TST) and Reserpine induced hypothermia. It has been reported that TST is less stressful and has higher pharmacological sensitivity than FST.[20] The index of depression in FST and TST models is taken as the immobility duration over a specific period of time. This immobility reflects helplessness or despair behaviour of animal equivalent to depression in human beings. The increase in immobility period indicates the depressed state of mind. Whereas, the reduction in immobility period reflects a depression-free state of mind. In the present study, different concentrations of Ananas cosmosus juice, when administered for 15 successive days to mice decreased significantly the immobility duration in both (TST and FST) the experimental models. These experimental models are precise and effective in predicting the anti-depressant potential of different categories new drugs.[17] Hypothermia or decreased body temperature is associated with the state of depression. Whereas, the increase in the body temperature or hyperthermia, is linked with a depression-free state of mind.[20] Depletion of biogenic amines (noradrenaline, 5-hydroxytryptamine, dopamine) in the brain induces hypothermia in rodents. Reserpine is an established pharmacological tool widely employed to deplete catecholamines and serotonin. The subcutaneous administration of reserpine leads to a decrease in rectal temperature of rats within 18 h of its administration. The hypothermia produced by reserpine was antagonized by classical anti-depressants.[21] ACJ, when administered for 8 successive days to rats (another species), reversed the hypothermia induced by reserpine. This effect of ACJ was comparable to marketed anti-depressants viz imipramine (Tricyclic anti-depressant) and fluoxetine (5-HT reuptake inhibitor). These findings highlight the fact that Ananas cosmosus juice possessed useful anti-depressant activity.

Noradrenaline and serotonin levels are diminished considerably in patients suffering from depression.[22] Since, Pineapple contains high amounts of serotonin[6] and fair amounts of noradrenaline[7],

there is a possibility that the low levels of both, noradrenaline and serotonin observed in depressed patients could be replenished by Pineapple juice. Prazosin (α-1 adrenoceptor antagonist)/sulpiride (selective D2-receptor antagonist)/p-CPA (serotonin antagonist), when administered on day 15, after the oral feeding of ACJ antagonized the effect of AC Juice in TST model on immobility duration of mice. This observation suggests that the anti-depressant effect of Pineapple juice is mediated via either α-1 adrenoceptors, dopamine D2 receptors or serotonergic receptors. MAO regulates the metabolic degradation of catecholamines, serotonin and other endogenous amines in CNS.[23] Since, noradrenaline and serotonin are metabolized by MAO-A and MAO-B enzymes, inhibition of MAO enzyme (MAO-A as well as MAO-B) would lead to enhanced levels of both noradrenaline and serotonin, thereby rectifying the deficiency of these amines responsible for producing depression. Both, Ananas cosmosus juice and phenelzine (standard MAO inhibitors), uniformly reduced significantly the brain MAO-A and MAO-B activity as compared to the control group. Therefore, Ananas cosmosus juice may be exploited clinically for the management of depressive disorders. Free radicals are responsible for producing neuronal damage in the body. The generation of free radicals is usually increased during stressful situations and illness. Malinoaldehyde (MDA) levels are taken as an index of free radical generation. Pro-oxidant/antioxidant balance is crucial in neurodegenerative processes, including cell death, motor neuron disease and axonal injury.[24] Increased MDA levels correspond to increased generation of free radicals leading to brain damage, whereas, decreased MDA levels reflect reduced free radical generation leading to neuro-protection. In the present study, malondialdehyde (MDA) levels were significantly reduced by Ananas cosmosus juice, thereby indicating reduced generation of free radicals in the brain and producing ultimately neuro-protective effect.

CONCLUSION

The Pineapple juice produced powerful and consistent anti-depressant effects in all the experimental models *viz* tail suspension test, forced swim test and reserpine induced hypothermia in the present study. It is remarkable to note that Pineapple juice contains high amounts of neurotransmitter serotonin and fair amounts of neurotransmitter noradrenaline, which play an important role in the pathology of depression. Furthermore, MAO inhibitory property and anti-oxidant activity possessed by Pineapple might be contributing favorably to the anti-depressant potential. Thus, it is worthwhile to investigate clinically the usefulness of Pineapple in managing depressive disorders.

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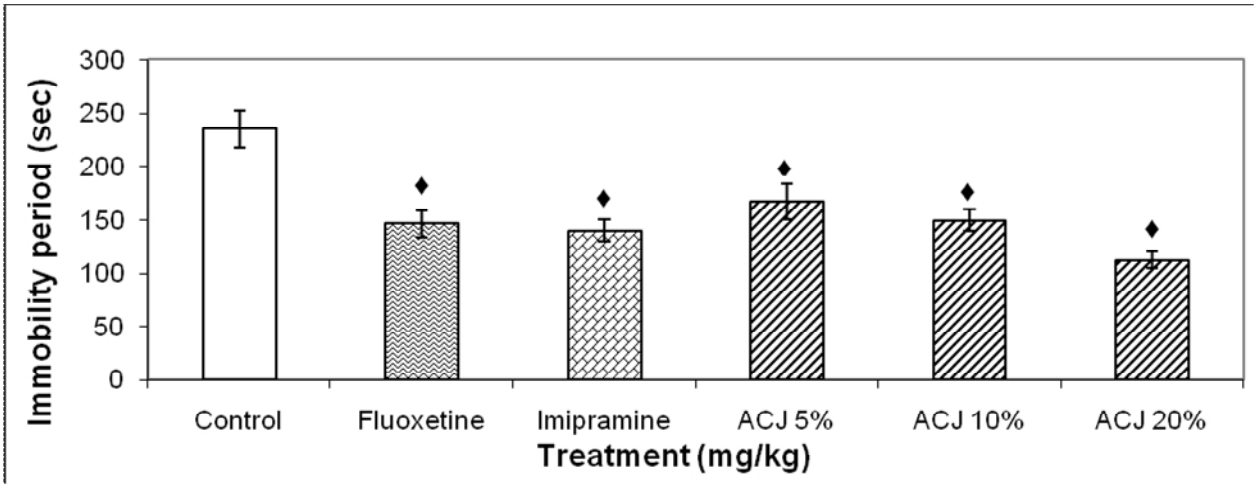

Figure 1: Effect of various concentrations of *Ananas cosmosus* Juice (ACJ 5, 10 & 20% v/v) administered orally for 15 successive days on Immobility Period in mice subjected to Tail Suspension Test

Fluoxetine (20 mg/kg, p.o.) and Imipramine (15 mg/kg, p.o.) were used as standard drugs.
Values are in Mean ± SEM. (n=6).
♦ denotes $p<0.01$ as compared to control group **ACJ** = Ananas cosmosus juice
One way ANOVA followed by Dunnett's t-test.

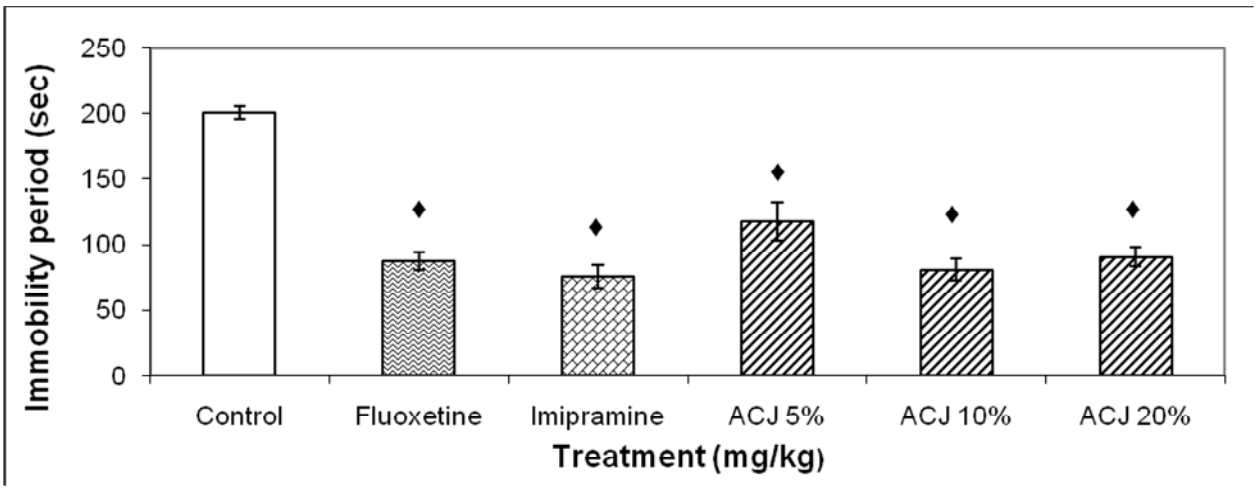

Figure 2: Effect of various concentrations of *Ananas cosmosus* Juice (ACJ 5, 10 & 20% v/v) administered orally for 15 successive days on Immobility Period in mice subjected to Forced Swim Test

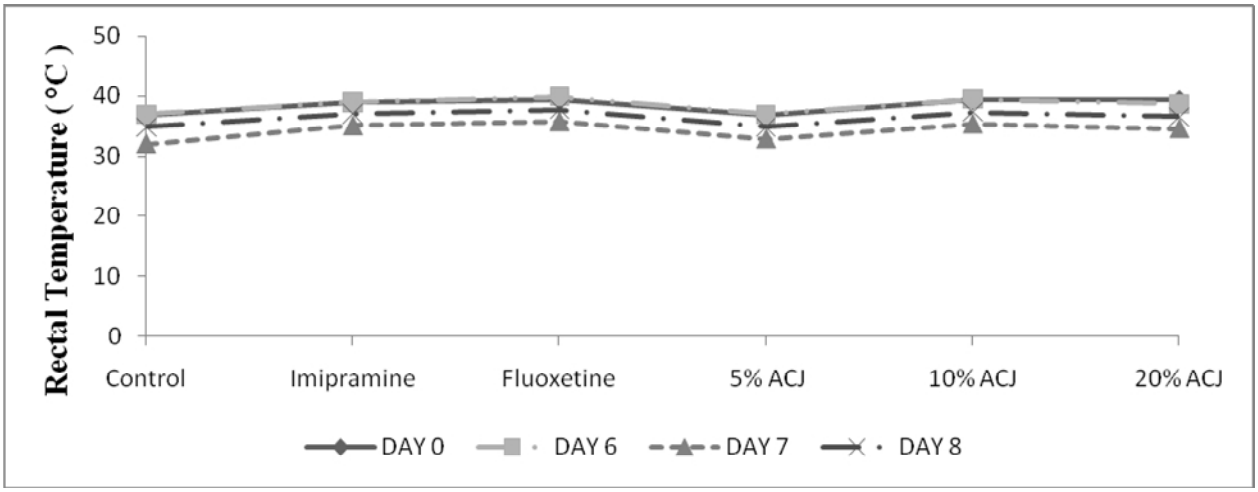

Figure 3: Effect of various concentrations of *Ananas cosmosus* Juice (ACJ 5, 10 & 20% v/v) administered orally for 8 successive days on body temperature in rats subjected to Reserpine induced hypothermia Test

Fluoxetine (20 mg/kg, p.o.) and Imipramine (15 mg/kg, p.o.) were used as standard drugs.
Values are in Mean ± SEM. (n=6).
One way ANOVA followed by Dunnett's t-test.

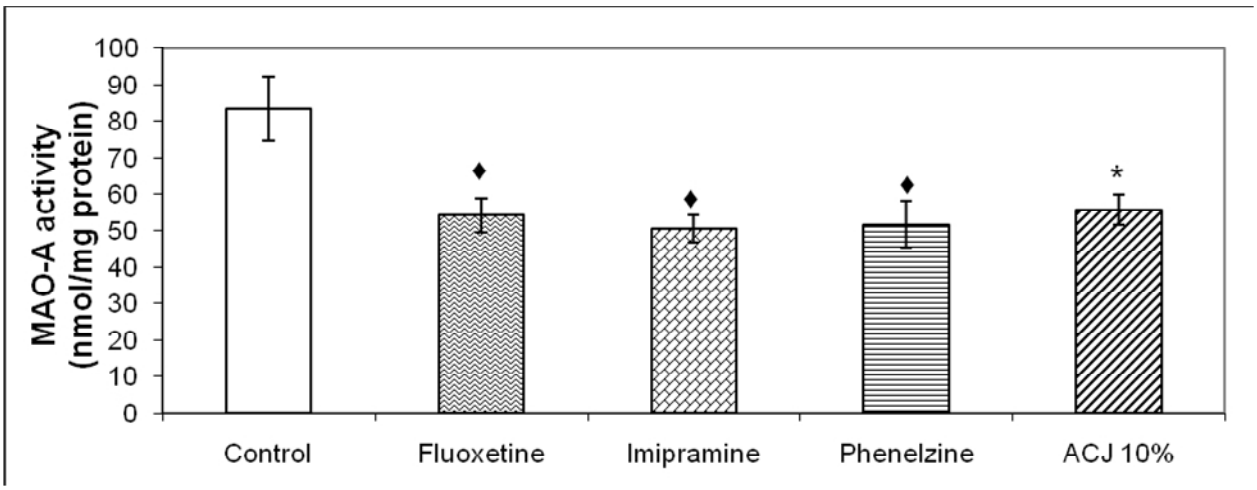

Figure 4: Effect of *Ananas cosmosus* Juice (ACJ 10% v/v) administered orally for 15 successive days on MAO-A activity in mice

Fluoxetine (20 mg/kg, p.o.), Imipramine (15 mg/kg, p.o.) and Phenelzine (20 mg/kg, p.o.) were used as standard drugs.
Values are in Mean ± SEM. (n=6).
* denotes $p<0.05$ & ♦ denotes $p<0.01$, when compared to control group.
ACJ = Ananas cosmosus juice
One way ANOVA followed by Dunnett's t-test

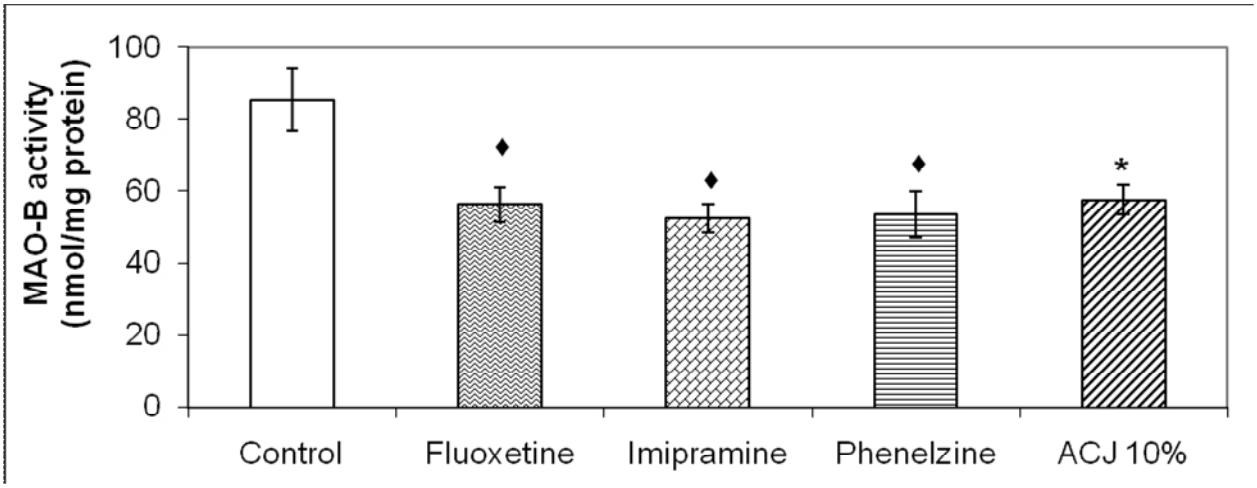

Figure 5: Effect of *Ananas cosmosus* Juice (ACJ 5, 10 & 20% v/v) administered orally for 15 successive days on MAO-B activity in mice

Fluoxetine (20 mg/kg, p.o.), Imipramine (15 mg/kg, p.o.) and Phenelzine (20 mg/kg, p.o.) were used as standard drugs. Values are in Mean ± SEM. (n=6).

* denotes $p<0.05$ & ♦ denotes $p<0.01$, when compared to control group.

ACJ = Ananas cosmosus juice

One way ANOVA followed by Dunnett's t-test

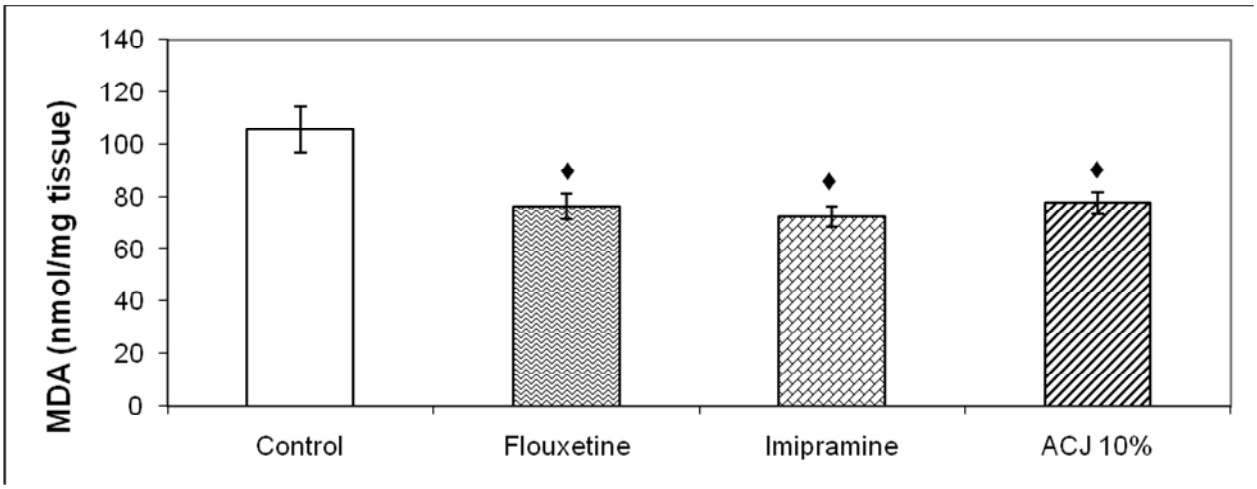

Figure 6: Effect of *Ananas cosmosus* Juice (ACJ 5, 10 & 20% v/v) administered orally for 15 successive days on MDA levels in mice

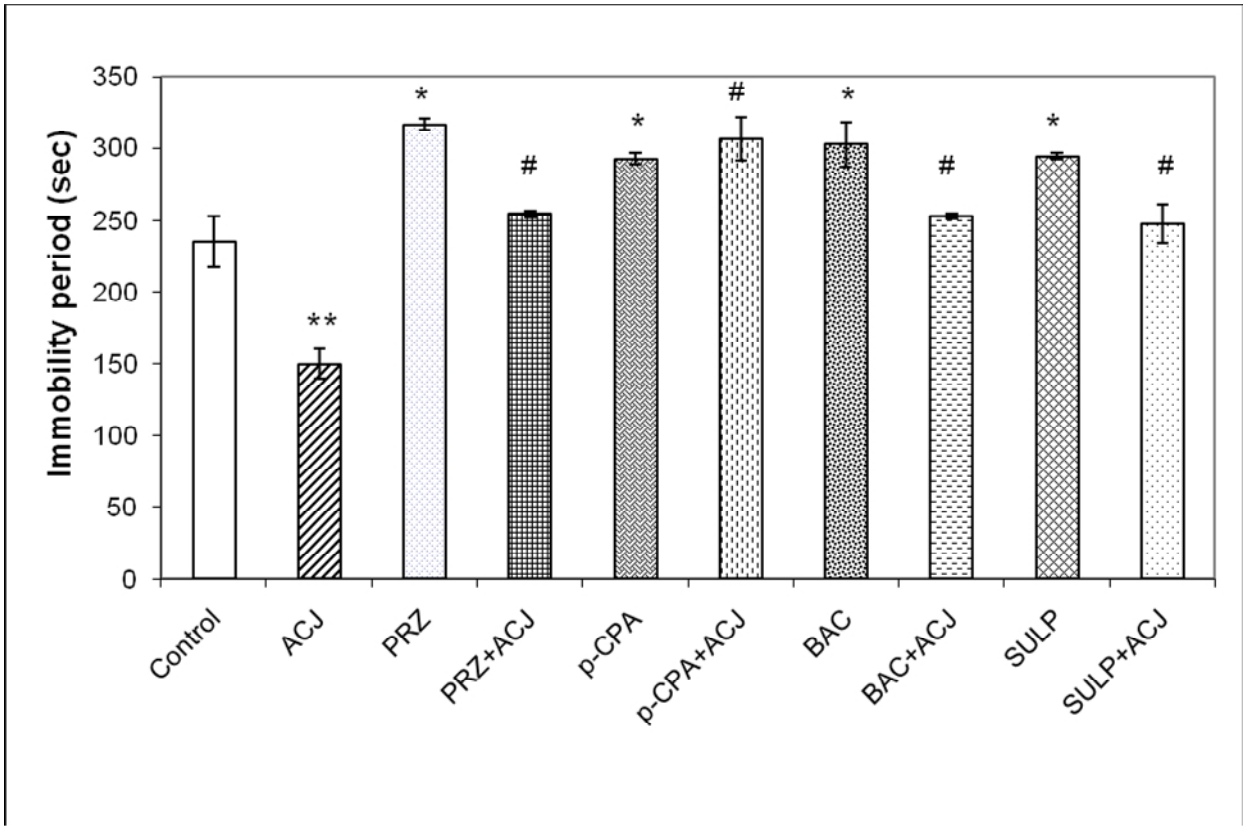

Figure 7: Effect of combination of *Ananas cosmosus* Juice (ACJ 10% v/v, p.o.) with prazosin/p-CPA/baclofen/sulpiride administered orally for 15 successive days on immobility period in mice subjected to Tail Suspension Test.

Prazosin (62.5 mg/kg, i.p.), p-CPA (100 mg/kg, i.p.), baclofen (10 mg/kg, i.p) and sulpiride (50 mg/kg, i.p) were used as standard drugs.

Values are in Mean ± SEM. (n=6).

* denotes $p<0.05$ and **denotes $p < 0.01$, when compared to control group

# denotes $p < 0.01$, when compared to ACJ alone.

ACJ = *Ananas cosmosus* juice, **PRZ**= Prazosin , **p-CPA**= para chlorophenyl Alanine,

BAC= Baclofen and **SULP**= Sulpiride

One way ANOVA followed by Dunnett's t-test.

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