ANTIDEPRESSANT ACTIVITY ON METHANOLIC EXTRACT OF *ANANAS COMOSUS* LINN PEEL (MeACP) BY USING FORCED SWIM AND TAIL SUSPENSION APPARATUS IN MICE

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ABSTRACT: Background: *Ananas comosus Linn is famous in traditional medicine for its abortificant and anti inflammatory effects. Its peel is already evaluated and established as a remarkable antioxidant agent. Despite its intensive use in number of conditions, its neuropharmacological studies are still missing. So this study was performed (1) to analyze the qualitative phytochemical composition of methanolic extract of Ananas comosus Linn peel, and (2) To evaluate the antidepressant-like effects at different doses.* ***Methodology****: Phytochemical screening of MeACP was performed by using standard chemicals and methods. 60 NMRI mice of either sex were randomly divided into Control group which received 5% Tween 80, a standard group which received Imipramine (15mg/kg) and three treatment groups which were given three doses 3.25, 7.5 and 15 mg/kg doses of MeACP. Antidepressant effects were measured in the forced swimming and tail suspension tests. Each group consisted of 6-7 animals.* ***Result:*** *All three test doses of MeACP (3.25, 7.5 and 15 mg/kg) induced antidepressant-like behavior in FST and TST ($p<0.001$) and comparable with positive control Imipramine 15mg/kg.* ***Conclusions:*** *Methanolic extract of MeACP possess excellent antidepressant potential at low doses but its effect on long term administration and its safety profile in acute and chronic administration is needed to be further evaluated.*

Key Words: Ananas comosus Linn, Forced swim test, Tail Suspension test, Imipramine, Flavonoids, Phenols.

INTRODUCTION

The number of individuals handicapped by a mental conditions has expanded by five folds following 1955, and the number is expanding by 400 individuals a day[1]. Among these mental illnesses, major depression is one of the, intermittent mental issues connected to diminished functioning, quality of life, morbidity, and mortality. They were the second driving reason for (years of life lived with disability) YLDs in 2010[2, 3] World Health Organization has rated depression as the fourth considerable reason for incapacity worldwide and its hypothesized that by 2020 it will become second leading cause of disability.[4] Pakistan is considered amongst the most crowded nations, whose populace is Approx 185 million [5], and has only 1% of yearly health financial plan. Moreover psychological well-being does not have an allocated budget at all [6]. Major reason behind psychological disability in Pakistani population is sociopolitical insecurity, financial vulnerability, savagery, territorial clash, and displacement for about previous three decades [7]. Because of the big percent of the masses that experience social misfortune incidence of depressive cases in Pakistan may be higher than other developing regions [8].

As pathophysiology of depression is quite complex, clinical usefulness of conventional antidepressant drugs has been limited not only due to undesirable reactions but also due to ineffectiveness of these agents [9]. Thus these disappointments in a few depressive conditions are the purposes behind nonstop scan for more up to date agents for depression. Henceforth it becomes obvious to investigate natural plant sources as effective antidepressant agents with minimum side effects [9, 10]. Majority of the population from sub-continent normally is more inclined towards the utilization of herbal medication over the conventional medication for their treatment, because of its cost adequacy in addition to fewer side effects [11]. Furthermore it is reported by another WHO that 33% of the world's population has no consistent access to advanced medicine [12]. Therefore plant based medications came into utilization in the modern medicine through the use of plant material as indigenous cure or conventional frameworks of medicine [13].

Pineapple, is the main tropical and nutritive fruit from family Bromeliaceae [14]. Whose scientific name is *Ananas comosus* Linn. Pineapple fruit along with other parts of the plant have strong medicinal properties and has a well established place in conventional system of medicine [15]. Fruit and some other parts of the plant have already explained for their pharmacological potential but when it comes about its peel evaluation it remains in scar, especially its CNS activity. To our best knowledge this is the first report on the antidepressant activity of Me-ACP. *Ananas comosus* Linn peel is already reported for its bioactive components like flavonoids and Vit-C in considerable amounts [16]. Present study focuses on utilization of AC peel in neuropsychiatric conditions characterized by of deprived Serotonin and Norepinephrine, like depression and anxiety, as *Ananas comosus* Linn plant is thought to be rich in serotonin and nor epinephrine [17].

MATERIAL AND METHOD

Plant material

The fruit of *Ananas comosus* Linn (Pineapple) was collected from the local fruit market of Karachi, Pakistan and was authenticated by Dr. Rubina Dawer, Department of Botany, University of Karachi. A voucher specimen was deposited in the Herbarium of Department of Botany, University of Karachi with specimen # 86522.

Animals

Experimental animals include mice (NMRI) of either sex weighing 20-30 grams for all the experiments. Each experimental group of animals contained 6 mice. All the experiments were conducted in a special noise free dim lit room. Each animal was used only once in the experiment.

Drugs and chemicals

5% Tween 80 from VWR International Ltd. England in Normal Saline (NaCl; Extra pure USP-BP) from Scharlar S.A. LaJota 86-08016- Barcelona, Spain used as control or

solvent for extract dilution. Imipramine – HCl (15 mg; Novartis, Pakistan) was used as standard antidepressant drug was purchased from local pharmacy.

Grouping of Experimental Animal

60 Animals were randomly assigned to different treatment groups which included Control Group which received 5% Tween 80, Positive Control Group that received Imipramine (15mg / kg) p.o as Known anti-depressant agent) and three test groups of MeACP with the doses of 3.25, 7.5 and 15 mg / kg for antidepressant activity.

Sample preparation

Peels of fruits was carefully removed, washed and then shade dried for 24 hours. The dried peel was powdered by laboratory mill and coarse fibrous material was obtained that was around 800 grams. This was extracted with Methanol (100%) thrice for 24 hours each (72 hours). Finally all three filtrates were combined and subjected to evaporation under vacuum using rotary evaporator, concentrated and then allowed for natural evaporation in fuming hood. After about one week the entire Methanol evaporated leaving behind semi solid gummy and oily mass (129 grams) which was 16.13% yield. The extract was transferred in a vial and store for future testing.

Phytochemical screening

The MeACP was qualitatively tested for the detection of carbohydrates (Molish test), saponins (Foam formation test), flavonoids (Lead acetate test), tannins (Ferric chloride test), alkaloids (Mayer's reagent), glycosides (Keller kiliani's test), reducing sugars (Fehling's test), and steroids (Salkowski test and Liebermann-Burchard test) following standard procedures.[18, 19]

Protocols for Antidepressant evaluation:

Forced swim test:

Antidepressant activity is evaluated by Forced swim test designed by Porsolt et al (1977) [20]. The test was performed according to the method of Ishaq et al [21] with slight modifications. It comprises of two sessions. Pretest session of 10 minutes was performed 24 hours before the test session which was of 5 minutes. Mice were subjected to swim independently in a glass chamber for 10 minutes than it was removed from the chamber, dried with a towel and sent back to their home cages. After 24 hours a 5 minutes test session was performed on each mouse [21]. Behaviors noted were climbing (upward movements with fore paws along the sides of the chamber), swimming (horizontal movements along the swim chamber or at whatever point mouse crossed the swim chamber to the next quadrant) [22], immobility time (sec) [23]. Each mouse was considered viewed as fixed immobile when it stopped battling and stayed gliding still in water, making just the efforts to keep its head above water [10]. Test drug, vehicle (5% Tween 80), and imipramine were administered three times a day i.e.15 minutes after the pretest, 18 and 1 hour prior to the test session [21]. Diminishing in the length of immobility time in the test session contrasted with the control group was considered antidepressant impact of the substance tested [24].

Tail suspension test:

Potential antidepressant effects of any test agent can also be evaluated by the use of "Tail suspension apparatus" [25]. The test was based on the fact that when animals are suspended by their tails they are subjected to the inescapable short-term stress and develop an immobile state. Dugs with antidepressant potential promote the appearance of escape-related behavior and reverse the immobility [26] The subjects were individually suspended 50cm above the floor by help of tape placed more or less 1cm from the tip of the tail [27]. Immobility duration was recorded for 5 minutes after 1st minute's, acclimatization time[28]. Absence of any limb body movements were characterized as Immobility, respiratory developments was the main exemption. Conventional anti depressant agents decrease the immobility time in this test[29].

Statistical analysis:

The statistical analysis was done on SPSS 19. The data obtained in experimental groups were evaluated by one-way analysis of variance (ANOVA) followed by LSD. The results were expressed as mean ± SEM. Values of $P \leq 0.05$ were considered significant.

RESULTS

Phytochemical screening

Phytochemical screening of Methanolic extract of *Ananas comosus* Linn peel shows that it contains alkaloids, flavonoids, saponins, tannins, steroids, cardiac glycosides and carbohydrates. (Table 1)

Table 1. Phytochemical constituents of Methanolic extract *Ananas comosus* Linn (MeACP)

Phytochemical Constituents	Tests	MeACP
Carbohydrates	Molish Test Fehling's test	+++ +++
Alkaloids	Mayer's Test	+++
Flavonoids	Lead acetate test 1 % Alcoholic $AlCl_3$	+++ +++
Saponins	Foam formation	++
Steroids	Salkowski test Liebermann-Burchard test	- ++
Tannins	Ferric chloride	++
Cardiac Glycosides	Keller kiliani's	+
+ Detected/Present, - Absent		

Effect of acute (single) treatment of different doses of MeACP in Forced Swim Test (FST):

In the forced swim test 3.25, 7.5, 15, mg/kg doses of MeACP were tested, with major parameters swimming, climbing and immobility time. Most important parameter, Immobility time was found significant when ANOVA was applied. {$F = 33.097$ (4, 25) $p<0.001$}. (Figure# 1) Multiple comparisons by Post hoc LSD reveals that immobility time is significantly reduced in treatment groups when compared with control group ($p<0.001$). Comparisons of MeACP treatment doses 3.25, 7.5mg/kg were also found highly significant with imipramine group ($p<0.001$) ANOVA also indicated highly significant Swimming behavior {$F= 59.299$ (4, 25) $p<0.001$} and climbing behavior {$F= 17.801$ (4, 25) $p< 0.001$} within the groups. IMP as a positive control drug in comparison with

vehicle group gave highly significant results in climbing behavior ($p< 0.001$), significant decrease in mobility time ($p<0.001$) but changes in swimming behavior were found insignificant. (Figure# 2 and 3)

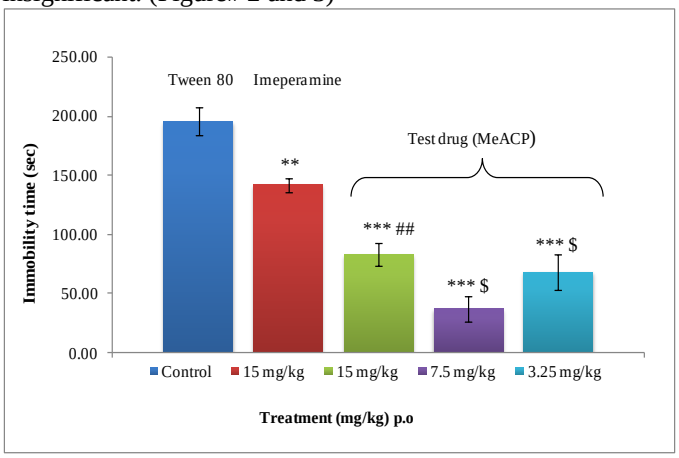

Figure 1. Effect of Imipramine and methanolic extract of Ananas comosus peel (MeACP) on immobility time of mice in Forced Swim test.

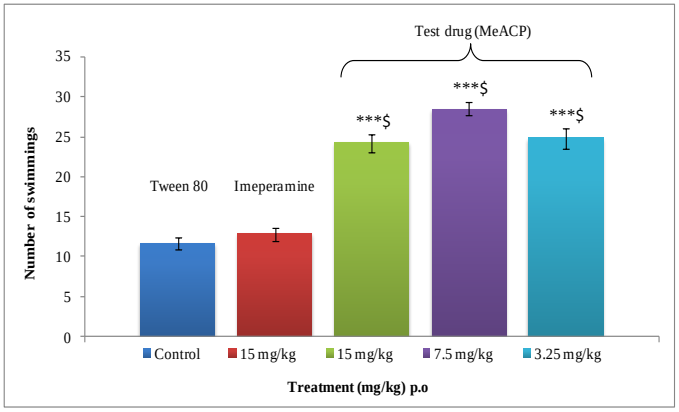

Figure 2. Effect of Imipramine and methanolic extract of Ananas comosus peel (MeACP) on swimming behavior of mice in Forced Swim test.

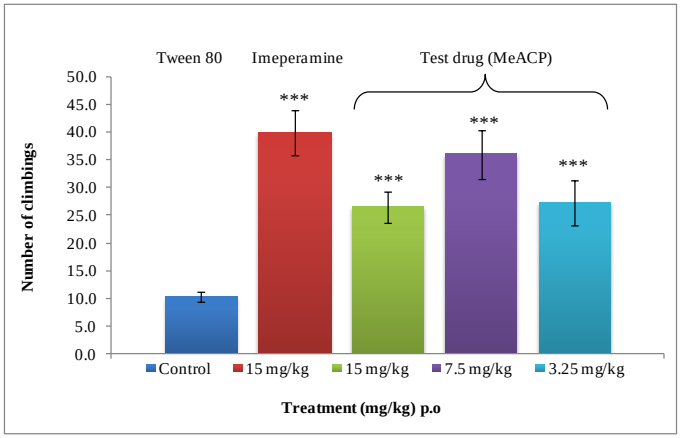

Figure 3 Effect of Imipramine and methanolic extract of Ananas comosus peel (MeACP) on climbing behavior of mice in Forced Swim test.

Figure 1, 2 and 3- Values represented as Mean ± SEM. n=6-7 (number of animals)* $p< 0.05$ = Significant, ** $p<0.01$ = More significant, *** $p<0.001$= highly significant compared to group treated with D.W plus Tween 80 (5%), $^{\#}$ $p< 0.05$, $^{\#\#}$ $p<0.01$, $^{\$}$ $p<0.001$ compared to the Imipramine group. Following ANOVA and Post hoc LSD df (4, 25)

Effect of acute (single) treatment of different doses of MeACP in Tail Suspension Test (TST):

One way ANOVA showed statistically significant differences in the duration of immobility {$F =49.89(4, 25)$ $P<0.001$} in the tail suspension test. Post hoc LSD showed that MeACP at 3.25, 7.5, 15 mg/kg significantly reduced the immobility in mice ($P<0.001$) vs. vehicle group. 3.25 and 7.5 mg/kg doses of MeACP were found more effective than IMP in reducing immobility. ($p< 0.001$)In the following test imipramine 15mg/kg also produced marked reduction in the immobility compared to vehicle group ($P< 0.001$). (Figure 4)

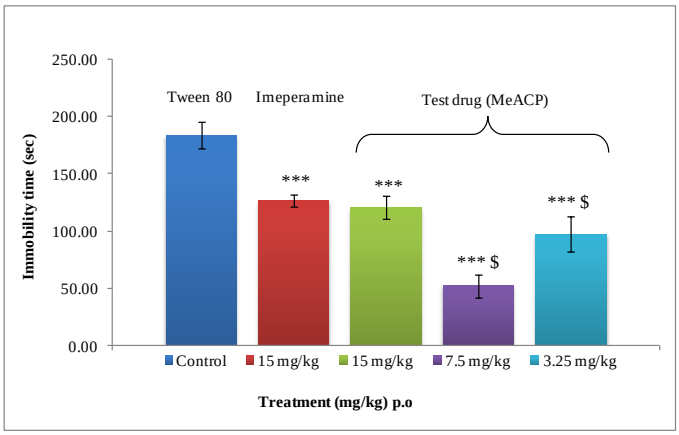

Figure 4. Effect of Imipramine and methanolic extract of Ananas comosus peel (MeACP) on immobility time of mice in Tail Suspension test

Figure 4- Values represented as Mean ± SEM. n=6-7 (number of animals) * $p< 0.05$ = Significant, ** $p<0.01$ = More significant, *** $p<0.001$= highly significant compared to group treated with D.W plus Tween 80 (5%), $^{\#}$ $p< 0.05$, $^{\#\#}$ $p<0.01$, $^{\$}$ $p<0.001$ compared to the Imipramine group. Following ANOVA and Post hoc LSD df (4, 25)

DISCUSSION

Depression is an emotional disorder causing deterioration of abilities and routine activities [30]. It is one of the most important causes of disability worldwide. Pathophysiological basis of depression is depletion of the neurotransmitters serotonin, norepinephrine or dopamine in the central nervous system [31]. Increased lipid peroxidation and reduced total brain antioxidant activity is also found to be associated with depression in different cases [32].

Qualitative analysis of *Ananas comosus* Linn peel extract revealed that it contains Carbohydrates, Alkaloids, Flavonoids, Saponins, Steroids, Tannins, Phenols and Glycosides. Alkaloids have already been reported to have remarkable effects on CNS.[13, 33] Presence of these constituents in MeACP provides support for its CNS based effects [34-36]. Its noteworthy antioxidant properties has already been claimed, as it is stated rich in antioxidants and

phenolic compounds [37, 38], such as myricetin, salicylic acid, tannic acid, *trans*-cinnamic acid, *p*coumaric acid [39], chlorogenic acid [40], rutin [41], naringenin [42], Quercitrin and kaempferol [40]. Quercitrin and kaempferol are found as main anxiolytic and antidepressant flavanoids in many studies[41, 43]. Additionally presence of Saponins in methanolic extract of AC are also reported by some researchers [44], make it all the more encouraging possibility to use the agent in conditions like depression[45].

FST and TST, both behavioral misery models are developed in an effort to mimic human stress [46]. Conditions normally seen in depressed animals are reflected as Immobility; a behavioral despair similar to human depression showing hopelessness[47].When *Ananas comosus* Linn peel extract was tested for antidepressant activity, results provided promising confirmation regarding oral administration that produced significant antidepressant like effect in TST and FST after single treatment. Nowadays it is extensively accepted that decreased brain monoamine neurotransmitters are linked with etiology of depression [48, 49] and to effectively treat it, these neurotransmitters should be maintained to normal or near normal levels[50].

Ananas comosus Linn is reported to contain numerous brain monoamines namely, serotonin, norepinephrine and dopamine [51, 52] thereby contributing positively in its antidepressant effect. After the single TDS treatment, MeACP significantly reduced immobility time at all three doses viz. 15mg/kg, 7.5mg/kg and 3.25mg/kg in the FST.($p<0.000$) Most interestingly all three doses of MeACP produced immobility more prominent than positive control (IMP) which is one of the standard drugs in antidepressant activity.($p<0.000$) IMP acts by blocking the reuptake of nor-adrenaline, dopamine and serotonin thus leads to an increase in the neurotransmitter levels[53].

Here we can specify the flavonoids that are reported to be present in *Ananas comosus* Linn like rutin, qurecitin and naringinin that were found active in different phyto pharmacological studies have antidepressant effects in FST and TST. Therefore, it can be said that antidepressant effect of MeACP is due to the presence of these phytochemicals [41, 48, 54, 55]. Decreased immobility induced by all doses of MeACP is evident for increased motivational behavior and lowered helplessness in investigational mice represents its antidepressant potential even better than IMP($p<0.000$). Other two parameters that are observed and found statistically significant for MeACP are Climbing and Swimming behaviors. Improvement in active behaviors is indirect indicators of biochemical pathway involved in anti depressant effect[45, 56]. imipramine which is rapidly converted to desipramine decrease immobility time [57] increases climbing behavior without effecting changes in swimming behavior [58]. This hypothesis is in agreement with our results of IMP in which immobility is decreased with increase in climbing behavior.

It has been verified that swimming is responsive to serotoninergic compounds, such as the selective serotonin reuptake inhibitor fluoxetine, and that climbing is responsive to tricyclic antidepressants and drugs with selective effects on catecholamine transmission [58]. The additive effect of MeACP on swimming and climbing attempts may suggests that *Ananas comosus* Linn peel extract at all given doses is affecting both the mechanisms. It not only alters the serotonergic pathways but also the catecholamine transmission. This can be linked with its multi phytochemical state as well as presence of serotonin and catecholamine [17].

Likewise in TST, MeACP and IMP produced a marked decreased in immobility time at all three doses same like as FST, marked decrease in immobility time statistically more significant than IMP. According to recently available data that instability in oxidative metabolism are associated with high anxiety and depression levels, thus for the prevention or treatment of these aliments the use of antioxidants has been suggested as a novel approach [43]. It is already explained that brain malfunction is linked with lipid peroxidation products and elevated total brain antioxidant activity [59, 60]. Polyphenols are naturally-occurring antioxidants found in many fruits which can have pharmacological effects on brain could be a successful option to deal with anxiety, depression and other diseases linked to oxidative stress [43, 61]. The methanolic extract yield of phenolic content of pineapple peel were 30.2% and 10mg/g GAE (Gallic acid equivalent) [37]. Further studies showed in a hepatotoxicity model that it has antioxidant effect and free radical scavenging properties [62].

The above researches clarify one of the possible mechanisms involved in antidepressant effect observed in this study, beside the existence of biologically active compounds present in *Ananas comosus* Linn peel extract with activities on CNS.

CONCLUSION

Our results demonstrated that methanolic extract of *Ananas comosus* peel possess pronounced antidepressant effect in mice FST and TST models of depression even at very low doses. Which was even more significant then IMP and shows a defined dose response pattern. Further evaluation of its mechanism of action and safety profile can give us a lead in depression treatment.

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