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Original Article

ANTIDEPRESSANT ACTIVITY OF AQUEOUS EXTRACTS OF FRUITS OF *TERMINALIA CHEBULA* AND *PHYLLANTHUS EMBLICA* IN BEHAVIOURAL MODELS OF DEPRESSION: INVOLVEMENT OF MONOAMINERGIC SYSTEM

PAWAR DATTATRAY B.*[1], MARATHE PADMAJA A.[2], REGE NIRMALA N.[3]

Medical Adviser, Alkem Laboratories Ltd, Alkem House, Senapati Bapat Marg, Lower Parel (West), Mumbai 400 013, India, [2]Professor Additional, [3]Professor and Head, Department of Pharmacology & Therapeutics, Seth G. S. Medical College & K. E. M. Hospital, Parel, Mumbai 400008. India.
Email: dr.datta_pawar@yahoo.co.in

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ABSTRACT

Objectives: *Terminalia chebula* (TCh) and *Phyllanthus emblica* (PE) posses wide range of central nervous system activity. This study was conducted to investigate the antidepressant activity and mechanism action of aqueous extract of TCh & PE using forced swimming test (FST) and tail suspension test (TST).

Methods: Aqueous extracts of TCh (390, 780 & 1560mg/kg) and PE (390, 780, 1560 & 3120 mg/kg) were administered for seven days to mice and immobility time was measured in FST & TST. Extracts were administered for 14 days and immobility time was measured in chronic FST. The mechanisms of antidepressant effect of TCh and PE were studied using prazosin (1mg/kg), levosulpiride (20mg/kg) and p-CPA (300mg/kg) in TST.

Results: In FST, 780 & 1560 mg/kg of TCh and 1560 & 3120 mg/kg of PE reduced immobility time while in TST, 1560 mg/kg of TCh and 3120 mg/kg of PE decreased immobility significantly compared to control. In chronic FST, only 780 & 1560 mg/kg of TCh showed significant reduction. Antidepressant effect of TCh was reversed by prazosin while antidepressant effect of PE was reversed by prazosin and levosulpiride significantly in TST.

Conclusions: The aqueous extracts of TCh and PE possess antidepressant activity at higher doses. This effect was possibly mediated through mono aminergic pathways.

Keywords: Animal models of depression, Forced swimming test, *Phyllanthus emblica*, Tail suspension test, *Terminalia chebula*.

INTRODUCTION

According to World Health Organisation, depression affects about 121 million people worldwide and it is among the leading causes of disability. By 2020, it would become the second leading contributor to global burden of disease in the world after cardiovascular diseases [1]. Currently, major groups of antidepressants used for treatment of depression include selective serotonin reuptake inhibitors (e.g. citalopram, sertraline, and Fluoxetine), tricyclic antidepressants (e.g. amitriptyline, imipramine and nortriptyline), monoamine oxidase inhibitors (e.g. phenelzine, moclobemide) and other newer heterocyclic antidepressants. The modern antidepressants are associated with untoward adverse effects including drowsiness, dry mouth, urinary retention, cardiac arrhythmias, gastro-intestinal upset, sexual dysfunction and they pose risk of overdose in vulnerable patients [2]. An estimated 29 to 46% of patients treated for major depressive disorder have either a partial response or no response at all to anti-depressive treatment [3]. Hence, there is a continued need to search for an antidepressant drug which is more effective and safe.

Terminalia chebula (TCh) and *Phyllanthus emblica* (PE) are claimed to possess wide range of central nervous system activity and are recommended for '*Mastishka Dourbalya*' (weakness of brain and nerves) which may be correlated with disorders like Alzheimer's disease, amnesia and dementia as per Ayurvedic text [4]. The extracts of the air-dried fruit of *T. chebula Retzius* (water, methanol, and 95% ethanol) have shown neuroprotective activity in experimental model of ischemic neuronal damage [5]. A polyherbal preparation BR-16 (Mentat) containing TCh and PE has been evaluated in models of anxiety and depression [6]. Literature search revealed that there is no experimental study reporting evaluation of antidepressant potential of single formulation of TCh. PE is advocated for a number of disease conditions in Ayurveda. It is widely used in the Indian subcontinent and is known to be safe on chronic consumption [7]. PE in the form of *Anwala churna* (an ayurvedic preparation) has been evaluated in models of memory and learning [8]. Earlier experimental studies have shown that hydro-alcoholic extract of PE (Gaertn) has also been tested for its antiepileptic activity [9]. Two groups of researchers have reported antidepressant-like effect of aqueous extract of fruits of PE in animal models of depression [10, 11].

Both TCh and PE are described as *rasayanas* (agents promoting longevity, resistance against infections and enhancing memory and intellect) in Ayurveda and have been shown to be useful as 'adaptogens' in animal models of stress [12-14]. Adaptogens help to counteract any adverse physical, chemical or biological stressor by generating non-specific resistance and allow the body to adapt to diverse demands imposed on it [14, 15]. Knowing the association of chronic stress and development of depression [16], the idea was conceived that adaptogen plants- TCh and PE may possibly have a role in therapy of depression.

Hence, in view of the above considerations, the present study was planned to evaluate antidepressant effect of TCh and also to strengthen the evidence about the antidepressant effect of PE; in behavioural models of depression in mice. It was also decided to find out mechanism of antidepressant activity of TCh and PE using monoaminergic antagonists.

MATERIALS AND METHODS

Animals used

After approval from the Animal Ethics Committee of the Institute, 216 Swiss albino mice of either sex (weighing 25-35 g) were procured and housed in the Central Animal House of the Institute maintained according to guidelines laid down by the Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA), India. The animals were housed in groups of 6 mice per cage in polypropylene cages with husk of paddy as the bedding. The animals were fed with food pellets (procured from Chakan Oil Mills, Maharashtra) and received filtered and UV purified water *ad libitum*. Temperature of the Animal House was maintained at 22 ± 3°C, with

50-70% humidity, with 12-15 filtered fresh air changes per hour and 12 hour light/dark cycles.

Study drugs used

The plant drugs selected for evaluation were standardized aqueous extracts of dried fruits of the two plants, TCh (extractive value- 45%) and PE (extractive value- 35%); purchased from *Natural Remedies Ltd.,* Bengaluru, India. The extracts were stored at room temperature in a desiccator containing sodium bicarbonate to avoid moisture. TCh extract contained 13.0% (w/w) of gallic acid, 49.2 % (w/w) and the moisture content was 4.7% (w/w). PE extract contained 11.5 % (w/w) of gallic acid and 36.8 % (w/w) of Tannins as tannic acid with moisture content of 2.6% (w/w). The doses of the extracts were derived based on the doses advocated for humans as per ayurvedic text [4, 17]. Three different doses of the TCh and four doses of PE were chosen for this study. Fluoxetine (Sun Pharmaceuticals Ltd., India) was selected as the positive control. TCh, PE and fluoxetine; dissolved in distilled water were administered orally once daily at the same time of the day (between 9.30 am to 11.30am) (Table 1).

Prazosin (alpha adrenergic antagonist) procured from Sun Pharmaceuticals Ltd., India; levosulpiride (selective D2 receptor antagonist) obtained from Intaas Pharmaceuticals, India - and p-chlorophenylalanine (serotonin synthesis inhibitor) purchased from Sigma-Aldrich, USA was used to study mechanisms of antidepressant effect of TCh and PE. Prazosin, levosulpiride and p-CPA were administered orally in distilled water.

Table 1: Part 1: Experimental groups for FST, TST and chronic FST

Group(N= 6)	Study Drugs	Dose (mg/kg/day)
1	Distilled water	0.5 ml
2	Fluoxetine	20
3	TCh -1	390
4	TCh -2	780
5	TCh -3	1560
6	PE -1	390
7	PE -2	780
8	PE -3	1560
9	PE -4	3120

TCh- *Terminalia chebula*, PE- *Phyllanthus emblica*

Prazosin and levosulpiride were administered once 45 minutes before conducting tail suspension test and p-CPA was administered 3 times; 72, 48 and 24 hours prior to tail suspension test [18]. The study doses of drugs are shown in Table 2.

Table 2: Part 2: Experimental groups for study of mechanism of action of TCh and PE

Group(N=6)	Study Drugs	Dose
1	DW	0.5 ml
2	TCh -3	780 mg/kg
3	PE -4	3120 mg/kg
4	Prazosin+ DW	1mg/kg + 0.5 ml
5	Levosulpiride + DW	Levosulpiride + 0.5 ml
6	p-CPA + DW	300mg/kg + 0.5 ml
7	Prazosin + TCh -3	1 mg/kg + 780 mg/kg
8	Levosulpiride + TCh -3	20 mg/kg + 780 mg/kg
9	p-CPA + TCh -3	300 mg/kg + 780 mg/kg
10	Prazosin + PE -4	1 mg/kg + 3120 mg/kg
11	Levosulpiride + PE -4	20 mg/kg + 3120 mg/kg
12	p-CPA + PE -4	300 mg/kg + 3120 mg/kg

DW- Distilled water, TCh- *Terminalia chebula*, PE- *Phyllanthus emblica,* p-CPA- *p*-chlorophenylalanine.

Study Procedures

The study was divided into 2 parts. In the part 1, antidepressant effect of TCh and PE was evaluated in the models of forced swimming test (FST) and tail suspension test (TST). In part 2, mechanisms of antidepressant action of TCh and PE were studied using prazosin, levosulpiride and p-CPA in the model of TST.

Part 1- Antidepressant activity in forced swimming tests and tail suspension test

Forced swimming test

The mice were randomly divided into 9 groups (6 /group). Modified version of the forced swimming test (FST) was used in the study [19]. The study groups were administered the respective drugs: distilled water (vehicle control), fluoxetine (positive control), TCh (three groups) were and PE (four groups); orally for 7 consecutive days (Table 1). On day 6, pre-test session was conducted wherein mice were forced to swim for 15 minutes in a 25 cm tall, 12 cm diameter glass cylinder filled to 15 cm with water (temperature 25°C ± 3°C). After the 15 min pre-test session, mice were removed from the water, wiped with towels and placed under a warming lamp until dry. On 7th day, 60 minutes following administration of the study drugs, immobility time was noted in all mice for five minutes. Immobility was defined as the time spent by mice making only movements necessary to keep their head above water. The water in the glass cylinder was changed after each swimming session.

Open field test for measurement of locomotor activity [20]

Open field test (OFT) was conducted in the mice, 5 min before subjecting them to FST. The open field apparatus is made of wooden box (36 X 36 X 30 cm high). Its floor is divided into nine squares of equal dimensions (12 X 12 cm). For OFT, each mouse was placed in the centre of arena. The locomotor activity of each mouse was assessed by counting the number of line crossings and rearing frequencies in the open field apparatus for 5 minutes. The apparatus was cleaned after each assessment to eliminate the possibility of any odor clues left by the previously tested mice. The experiments were conducted in dark and sound proof room.

Tail suspension test [19, 21]

Fifty-four mice were randomly divided into 9 groups (6/group).Tail suspension test (TST) was conducted according to the procedure described by Steru *et al* [22]. The study drugs were administered to the respective groups orally for 7 consecutive days at the same time of the day (**Table 1**). On 7th day, 60 minutes following administration of the study drugs, immobility time was noted in TST over a period of six minutes. Mice were considered immobile only when they hung passively and were completely motionless.

Chronic forced swimming test

A method devised by Kitamura *et al*. [23] was used to conduct chronic version of the FST. The mice were randomly allocated to 9 groups (6/group) (**Table 1**).The study drugs were administered orally, once every day for 14 days and the mice were forced to swim in the glass cylinder for 6 minutes every day for 13 days. On the day of test (i.e. day 14 of study drug administration), one hour following the study drug administration; test session of 6 min was conducted. Immobility time was measured for six minutes. The water in the glass cylinder was changed after each swimming session.

Part 2- Mechanism of antidepressant action of TCh and PE using prazosin, levosulpiride and p-chlorophenylalanine alone and in combination with TCh in TST [18]

The study drugs were administered to the respective groups orally for 7 consecutive days at the same time of the day (**Table 2**). TCh and PE were administered in the highest doses which were found to be effective in part 1 of the study for a period of 7 days. Prazosin and levosulpiride were administered intraperitoneally once 45 minutes and p-CPA was administered intraperitoneally 3 times; 72, 48 and 24 hours prior to the test session. All the observations in part 1 and 2 of the study were made by a trained observer who remained unaware of treatment allocation.

Statistical Analysis

The results were expressed as mean ± standard deviation (SD) values which were compared using one way ANOVA followed by post hoc Tukey's test using the Graph Pad Instat version 3.06. A 'p' value of <0.05 was considered significant.

RESULTS

Part 1- Antidepressant activity of TCh and PE

Forced Swimming Test

Treatment of mice with fluoxetine produced a significant reduction ($p<0.01$) in the immobility time compared to vehicle control ($p<0.001$). TCh-1 group showed small reduction in immobility time compared to control. The two higher doses of TCh-2 and TCh-3 led to significant reduction in immobility time compared to vehicle control ($p<0.01$) and the mean values of immobility time in both these groups were comparable to those in the fluoxetine treated group. There was a trend towards decline in immobility time observed with increasing doses of PE in the four PE groups. However, only the two higher dose groups, PE-3 ($p<0.05$) and PE-4 ($p<0.001$) showed significant reduction in duration of immobility when compared to the control. The immobility time in both PE-3 and PE-4 groups was found to be comparable to that in the fluoxetine treated group (Table 3).

Table 3: Effect of TCh and PE on immobility time in FST

Groups n=6	Drugs	Duration of immobility in seconds (Mean + S.D)
1	D/W	204.67 ± 16.95
2	FLU	141.67 ± 11.59**
3	TCh- 1	179.00 ± 22.39 NS
4	TCh- 2	157.33 ± 28.25 * NS1
5	TCh- 3	154.00 ± 16.91 * NS1
6	PE- 1	212.33 ± 20.10 NS
7	PE -2	173.17 ± 28.53 NS
8	PE -3	168.67 ± 20.36* NS1
9	PE- 4	138.67 ± 16.40** NS1

D/W: Distilled water, FLU: fluoxetine, TCh: *Terminalia chebula*, *p<0.01, **p<0.001 vs D/W group, NS - Not significant vs D/W group, NS1- Not significant vs FLU group, One way ANOVA with post hoc Tukey's test.

Open field test

Treatment with fluoxetine, TCh and PE at all doses did not show any difference in the number of line crossings and rearing frequencies as compared to control group treated with distilled water (Table 4).

Table 4: Effect of TCh& PE on line crossing and rearing frequencies in OFT

Groups n=6	Drugs	Number of line Crossings (mean + S.D.)	Number of rearing frequencies (mean + S.D.)
1	D/W	85.83 ± 20.27	27.17 ± 14.61
2	FLU	64.67 ± 21.82 NS	16.83 ± 4.59 NS
3	TCh -1	78.83 ± 20.51 NS	23.00 ± 7.46 NS
4	TCh - 2	87.17 ± 18 NS	20.17 ± 6.08 NS
5	TCh -3	67.17 ± 17.96 NS	19.17 ± 4.36 NS
6	PE- 1	76.17 ± 15.48 NS	27.50 ± 9.01 NS
7	PE - 2	77.17 ± 19.21 NS	30.00 ± 10.66 NS
8	PE- 3	66.00 ± 11.83 NS	18.67 ± 4.50 NS
9	PE - 4	85.17 ± 5.81 NS	23.67 ± 5.47 NS

D/W: Distilled water, FLU: fluoxetine, TCh: *Terminalia chebula*, NS - Not significant vs D/W group**,** One-way ANOVA with post hoc Tukey's test.

Tail suspension test: As shown in **Table 5**, fluoxetine treated group caused a significant reduction in immobility time as compared to control ($p<0.001$). In TCh-1 and TCh-2 groups, there was no decrease in duration of immobility compared to control. TCh-3 group showed significant reduction in immobility time versus control ($p<0.01$). However, the reduction in immobility time in fluoxetine group was far more than that observed in the TCh-3 group. The PE-1, PE-2 and PE-3 groups did not produce any decrease in immobility time as compared to control. Although the PE-4 group showed significant reduction in immobility time compared to vehicle control ($p<0.001$), the extent of reduction was lesser when compared with that of fluoxetine group.

Table 5: Effect of TCh and PE on immobility time in TST

Groups n=6	Drugs	Duration of immobility in seconds (Mean + S.D.)
1	D/W	202.33 ± 21
2	FLU	62.83 ± 20.76**
3	TCh -1	194.83 ± 28.82 NS
4	TCh -2	159.67 ± 21.79 NS
5	TCh -3	136 ± 32.12 * †
6	PE -1	182.83 ± 32.82 NS
7	PE -2	167.00 ± 22.51 NS
8	PE -3	171.33 ± 17.14 NS
9	PE -4	133.67 ± 12.32* †

D/W: Distilled water, FLU: fluoxetine, TCh: *Terminalia chebula*, * p<0.01, ** p<0.001 vs D/W group. †p< 0.001 vs FLU group, NS- Not significant vs D/W group, One-way ANOVA with post hoc Tukey's test.

Chronic forced swimming test: Treatment with fluoxetine produced a reduction in immobility time (p<0.001). TCh-1 treatment did not cause any decrease in duration of immobility as compared to the control group. TCh-2 (p<0.05) and TCh-3 (p<0.001) groups showed significant reduction in immobility time compared to control which was significantly less than that observed in fluoxetine group. The three PE treated groups: PE-1, PE-2 and PE-4 showed no reduction in the duration of immobility as compared to the vehicle control. A small (statistically insignificant compared to control) decrease in immobility time was observed in PE-3 group (Table 6).

Table 6: Effect of TCh& PE on immobility time in chronic FST

Groups n=6	Drugs	Duration of immobility in seconds (mean ± S.D.)
1	D/W	229.17 ± 9.93
2	FLU	156.00 ± 23.23 ***
3	TCh -1	216.50 ± 13.92 NS
4	TCh -2	203.67 ± 11.45 * ††
5	TCh -3	192.67 ± 8.66 ** †
6	PE -1	218.00 ± 22.11 NS
7	PE -2	226.17 ± 24.19 NS
8	PE -3	201.17 ± 11.29 NS
9	PE -4	217.00± 11.85 NS

D/W: Distilled water, FLU: fluoxetine, TCh: *Terminalia chebula*, *p<0.05, **p<0.01, ***p<0.001 vs D/W group. †p< 0.01, ††p< 0.001 vs FLU group, NS – Not significant vs D/W group, One-way ANOVA used with post hoc Tukey's test.

Part 2-Mechanism of antidepressant action of TCh and PE

Based on the Part 1 results, the highest doses of TCh (TCh-3) and PE (PE- 4) were selected to elucidate mechanism of antidepressant action of TCh and PE. The vehicle control group showed immobility time of 202.33±21. TCh-3 group showed significantly less immobility time (136.00 ±32.12) compared to the control group (p <0.01). Prazosin, levosulpiride and p-CPA per se did not produce any change in immobility time as compared to vehicle control. Treatment of TCh-3 pretreated mice with prazosin caused increase in immobility time which was significantly more (181.67±14.40) as compared to TCh-3 group. TCh-3 with levosulpiride and TCh-3 with p-CPA did not produce any significant change in immobility time as compared to TCh-3 group (Table 7).

Table 7: Effect of combination of TCh with prazosin, levosulpiride and p-CPA on immobility time in TST in mice

Groups n=6	Drugs	Immobility time in seconds (mean ± S.D)
1	D/W	202.33 ± 21.00
2	Prazosin + D/W	214.17 ± 18.52 NS
3	Levosulpiride + D/W	191.00 ± 14.70 NS
4	p-CPA + D/W	222.33 ± 16.49 NS
5	TCh -3	136.00 ± 32.12*
6	Prazosin + TCh -3	181.67 ± 14.40†
7	Levosulpiride + TCh -3	154.33 ± 16.10 NS1
8	p-CPA + TCh -3	167.67 ± 17.55 NS1

D/W: distilled water, p-CPA: p-chlorophenylalanine, TCh-3: *Terminalia chebula-1560 mg/kg,* NS -Not significant vs D/W group, *p<0.01 vs D/W group, †p<0.01 vs TCh–3 group, NS1-Not significant vs TCh–3 group. Data are expressed as mean ± S.D.. One-way ANOVA used with post hoc Tukey's test

PE- 4 group showed significantly less immobility time of 133.67 ± 12.32 compared to the control group (p <0.001). Treatment of PE-4 pretreated mice with prazosin (166.00 ±11.80) and p-CPA (178.83 ± 17.06) (except levosulpiride); produced increased immobility times and the values were significantly higher as compared to PE-4 group (Table 8).

Table 8: Effect of combination of PE with prazosin, levosulpiride and p-CPA on immobility time in TST in mice

Groups n=6	Drugs	Immobility time in seconds (mean ± S.D)
1	D/W	202.33 ± 21.00
2	Prazosin + D/W	214.17 ± 18.52 NS
3	Levosulpiride + D/W	191.00 ± 14.70 NS
4	p-CPA + D/W	222.33 ± 16.49 *
5	PE -4	133.67 ± 12.32**
6	Prazosin + PE -4	166.00 ± 11.80†
7	Levosulpiride + PE -4	158.00 ± 19.42 NS1
8	p-CPA + PE -4	178.83 ± 17.06 ††

D/W: distilled water, p-CPA: p-chlorophenylalanine, PE-4: *Phyllanthus emblica*-3120 mg/kg, NS – Not significant, *p <0.05, **p <0.001 vs D/W group, † p <0.05, †† p <0.01, NS1- Not significant vs PE -4 group, One-way ANOVA with post hoc Tukey's test.

DISCUSSION

In the present study, three graded doses of TCh (the lowest and highest therapeutic dose extrapolated from human doses as per Ayurveda literature and two times the highest dose) and four different doses of PE (the lowest and highest therapeutic doses as per Ayurveda literature, two and four times the highest dose) were chosen for the study. Previous experimental studies with TCh have used the dose range of 50 mg/kg to 800 mg/kg [24, 25].

PE has been evaluated in the models of depression in the dose range of 0.8 mg/kg [10] to 400 mg/kg [11] in previous studies. A broader and higher dose range of both the plant drugs compared to previous studies were selected in the present in order to study effects appearing at higher doses and identify dose dependent action. The models of FST and TST which are widely used for screening of antidepressant drugs were selected in the present study [19, 26]. Open field test (OFT) is performed to test locomotor activity to rule out nonspecific stimulant effect of plant extracts as most antidepressant drugs do not increase locomotor activity in OFT [27, 28].

The results of the study showed that aqueous extract of fruit of TCh at the doses of 780 and 1560 mg/kg produced significant antidepressant effects in FST and chronic FST but it failed to show any effect at lowest dose (390 mg/kg). The highest dose of TCh (TCh-3) produced the most consistent effect in all models. The effect

of TCh-3 was significantly reversed by administration of prazosin indicating that the antidepressant effect of TCh may be mediated through noradrenergic transmission by interaction with alpha-1 adrenoceptors in brain. TCh effect does not appear to be mediated through dopaminergic and serotonergic systems because levosulpiride and p-CPA did not increase the immobility time in TCh pre-treated mice. Aqueous extract of fruit of PE showed antidepressant effect at higher doses (1560 and 3120 mg/kg) but failed to show any effect at lower doses (390 and 780 mg/kg). It was surprising that in chronic FST, there was no reduction in immobility with PE-4. West *et al* have hypothesized that behavior of immobility in a water cylinder reflects a learned habituation taking place in an environment that has become more familiar to animal which leads to decrease in immobility [29]. However, it is difficult to explain why we did not find similar habituating effect in TCh-3 and TCh-4 groups. It was observed that effect of PE was reversed by prazosin and p-CPA and not by levosulpiride. This finding suggests that PE possibly exerts its antidepressant effectby interacting with alpha-1 adrenoceptors and to increase in serotonin (5-HT) synthesis in the brain. The comparable observations related to line crossings and rearing frequencies among all the study groups in the OFT excluded the possibility of general stimulant activity of TCh and PE.

Conventionally, hypotheses on the pathophysiology of mood disorders are based on aberrant intra-synaptic concentrations of the neurotransmitters serotonin and norepinephrine. However, recent neuroimaging and post mortem morphometric studies have demonstrated selective structural and morphological (macroscopic and microscopic) changes across various limbic and non-limbic circuits in the brains of depressed patients. Stress and antidepressant treatment have an opposite effect on the intracellular signaling, transcription factors and target genes. An evolving new hypothesis in the pathophysiology and treatment of depression involves adaptation or plasticity of neural systems. Depression could result from an inability to make the appropriate adaptive responses to stress or aversive stimuli. It is possible that antidepressant treatment could oppose these adverse cellular effects, which may be regarded as a loss of neural plasticity, by blocking or reversing the atrophy of neurons and by increasing cell survival and function [30].

There is an evidence of derangement of oxidant and antioxidant defense systems in depression [30]. It is speculated that some phytoconstituents present in TCh and PE extracts having antioxidant and neoroprotective effects may also contribute to antidepressant mechanism [31- 33].

We compared our findings with other studies reported in literature. To the best of our knowledge, there is no study reported with TCh. In a study by Dhingra *et al* [11] the aqueous extract of fruit of PE showed decreased immobility period in both tail suspension test and forced swim test which was postulated to be due to interaction of PE with α_1-adrenoceptors, dopamine D_2-receptors, serotonergic, and $GABA_B$ receptors whereas our study results indicated possible interaction only with adrenoceptors and serotonergic receptors. Dhingra *et al* also observed a significant decrease in brain MAO-A levels in animals treated with PE [11]. In another study by Pemminati *et al* aqueous extract of fruits of PE was found to be more effective than imipramine in the models of TST and FST [10]. However in our study, although PE was effective at higher doses, the effect was smaller as compared to fluoxetine. The variations in responses obtained with PE in this study as compared to the previous two studies are difficult to explain but may be attributed to different doses used by us. Tannic acid is known to produce nonselective inhibition of monoamine oxidase, thereby increasing the levels of monoaminergic neurotransmitters in the brain [10]. This fact explains the effect of PE in decreasing brain MAO-A levels in study by Dhingra *et al* [11]. Facilitation of monoaminergic transmission through effect on MAO-A enzymes and / or decrease neuronal reuptake of serotonin could be other mechanisms mediating antidepressant effects of TCh and PE which were not explored in the present study. In the present study, we have shown the antidepressant effect of TCh for the first time. Our findings also corroborate with the evidence available for PE for its antidepressant potential. A wide dose range of TCh and PE in animal models of depression have been evaluated in this study for the first time. It is necessary to confirm safety profile of wide dose range of TCh and PEused in this study by carrying out detailed toxicity studies. It will be worthwhile to carry out further studies to confirm antidepressant effects of TCh and PE in other established models of depression and also study their interaction with modern antidepressants. The possible mechanism of antidepressant action of TCh and PE may be through modulation of monoaminergic pathways as shown in this study. Individual phyto-constituents of TCh and PE by virtue of their antioxidant and neurotropic properties may also be contributing to antidepressant effect and these properties need to be investigated further.

CONFLICT OF INTERESTS

Declared None

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