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Original article

## The role of serotonergic and catecholaminergic systems for possible antidepressant activity of apigenin



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## ABSTRACT

**Background and objective:** Although, the anti-depressant like effects of apigenin (APG) are documented in the literature, the underlying mechanism for exerting such an effect is still not clear. In this research, an attempt was made to determine the possible role of APG for antidepressant activity through serotonergic and catecholaminergic systems using standardized animal models.

**Materials and methods:** The antidepressant property of APG was determined by involving tail suspension (TST) and modified forced swimming tests (MFST). The effect of APG was evaluated at 25 and 50 mg/kg. In mechanistic models, animals were pretreated with catecholaminergic and serotonergic antagonists prior to administration of APG. The results obtained were statistically analyzed to determine the level of significance.

**Results:** The period of immobility in both models (TST and MFST) was significantly reduced by APG (25 and 50 mg/kg). The best therapeutic dose of APG (50 mg/kg) was selected for the mechanistic study. The anti-immobility effect of APG declined to a significant extent upon pretreatment with catecholaminergic antagonists ( $\alpha$ -methyl-para-tyrosine methyl ester; SCH 23390; sulpiride; phentolamine) and serotonergic inhibitors (*p*-chlorophenylalanine-methyl-ester; ondansetron) in both TST and MFST models. The antidepressant benefits of apigenin were only modestly reversed when rats were given propranolol.

**Conclusions:** The findings suggest that APG's antidepressant effect is mediated by the  $\alpha$ -adrenergic, dopaminergic and 5-HT<sub>3</sub> serotonergic receptors.

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## 1. Introduction

One of the commonly occurring mental ailment is depression seen across large number of people worldwide (Al Nasr et al., 2020). Major depressive disorder is a significant psychological condition marked by a persistent depressed mood, low self-esteem, and a loss of interest or pleasure in typically pleasurable activities (Weng et al., 2016). Depression differs from typical mood swings and emotional responses to life's challenges in that it lasts longer. The monoamine hypothesis of depression proposes a fall in 5-hydroxy tryptamine, noradrenaline and/or dopamine levels (Delgado, 2000). Published literature supports the involvement of catecholamines in modulating the antidepressant activity of sev-

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eral drugs (Elhwuegi, 2004). The significance of serotonin in the development of depression has also been confirmed by investigations of “tryptophan depletion,” that showed a fall in brain serotonin levels by reducing the availability of tryptophan (Smith et al., 1997). Antidepressants that has been developed in the last several decades were based on the monoamine hypothesis of depression. Nonetheless, pharmacological therapies for depression remain unsatisfying despite their wide spectrum (Berton and Nestler, 2006). To begin with, patients frequently require multiple antidepressant medications before responding to a treatment, and only around 65% of patients have some level of treatment efficacy (Duman, 2004). Second, weeks or months of treatment are required before a therapeutic response is reached; this therapeutic delay is significant since it has been linked to an elevated risk of suicide (Blier, 2003). Overall, depression's neurobiology and responsiveness to antidepressant medication are poorly understood. Therefore, it is imperative to promote credible research that has beneficial effects in controlling depression.

Natural products have long been employed as viable alternatives in the development of novel medications and therapeutic agents for a wide range of ailments. Apigenin (5,7,4'-trihydroxyflavone) is a well-studied phenolic and is one of the most widely distributed flavonoids in plants. Apigenin (APG) is found in a substantial proportion as glycosylated in daily eatables and nutritional drinks (Hostetler et al., 2017). APG has several interesting pharmacological actions and nutraceutical potential. Its antioxidant capabilities are widely recognized, and potential therapeutic activity to treat disorders like inflammation, autoimmune disease, neurodegenerative disease, and even cancer is documented (Salehi et al., 2019). APG inhibits the proliferation of hepatocellular carcinoma cells via up- or down-regulating miRNA molecules and their target genes (Wang et al., 2021). Further, a study reported the role of APG in inhibiting hepatic stellate cell activation and autophagy via the TGF-1/Smad3 and p38/PPAR pathways, which may help to reduce liver fibrosis (Ji et al., 2021).

A detailed understanding of APG's mechanism of action is crucial for future nutraceutical usage due to its vast spectrum of pharmacological effects and value to human health. Apigenin is known to have anti-anxiety and sedative effects (Avallone et al., 2000; Zanolini et al., 2000). A recent study documented muscle relaxant property of apigenin in animal experimental models (Asdaq et al., 2021). One of the published studies (Nakazawa et al., 2003a; 2003b) reported the involvement of dopaminergic system in potential antidepressant activity of APG. APG also decreased the activity of monoamine oxidase (MAO) (Han et al., 2007). The inhibiting action on MAO increases the level of monoamines such as serotonin in brain that is associated with the remission of depressive symptoms (Elhwuegi et al., 2005). There are number of reports that exhibited the inhibitory effects of APG on the functioning of gamma-aminobutyric acid (GABA) and N-methyl-D-aspartate (NMDA) receptors, and the antagonism of these receptors may lead to the relief of depression (Nakazawa et al., 2003a; 2003b; Skolnick, 1999). These findings suggest that APG's antidepressant characteristics are not mediated by a single mechanism of action, and that many neurotransmitters may be involved in its pharmacological activity. Therefore, this study was designed to assess the antidepressant effect of APG using established animal experimental models of tail suspension (TST) and modified forced swimming tests (MFST) in animals pretreated with serotonin and catecholaminergic antagonists.

## 2. Materials and Methods

### 2.1. Experimental animals

Albino mice (either sex) weighing 20–25 g were housed in an air-conditioned room following established system of maintenance

and research on animals. In order to avoid any impact on the behavioral pattern of the animals, uniform temperature, light and sound were maintained throughout the experimental period. The proposal of this study was granted approval by the institutional ethical committee.

### 2.2. Chemicals and drugs

“Beijing Mesochem Technology Co., Ltd. (Beijing, China)” provided apigenin (purity: 98.20 percent by HPLC). The rest of the chemicals required for this investigation were obtained from well-known suppliers, and the labels were recorded for future use. Ondansetron hydrochloride, p-chlorophenylalanine methyl ester,  $\alpha$ -methyl-para-tyrosine methyl ester (AMPT), R(+)-SCH-23390 hydrochloride, sulpiride, phentolamine hydrochloride, and propranolol were procured from Sigma-Aldrich. Sodium chloride (0.9 percent) was used for dissolving all compounds except AMPT, whereas, the solution of AMPT was made in 10% Tween 80. Apigenin (25 and 50 mg/kg was given orally (Redrobe et al., 1998) three times before the test sessions, at 24, 5, and 1 h (Shoubaky et al., 2016). The standard antidepressant fluoxetine (30 mg/kg, oral) (Castagné et al., 2011) was used to compare antidepressant activities. Except for SCH23390, which was given subcutaneously, all other compounds were given by intraperitoneal route (10 ml/kg).

### 2.3. Tail suspension test

The tail-suspension test is a behavioral test for mice that can be used to screen for prospective antidepressants. This experiment was carried out using the approach explained previously (Müller et al., 2012). In short, each mouse was taped 50 cm above the ground around a centimeter from the edge of the tail. The full length of non-mobility that is counted when animal remains hanged without any motion as well as resistance was noted during the 6-minutes of the experimental duration.

### 2.4. Modified forced swimming test

This test was done following a procedure described in another study (Steru et al., 1985). A 30 cm diameter cylinder was taken and mice were made to swim for 15 min one day before the experiment for training and 24 h later, the duration of swimming during recording time of five minutes were noted during experimental phase. The period of non-mobility, swimming and climbing were recorded. The mouse's immobility period was defined as the time it spent floating in the water without resisting and making just the movements required to keep its head above water.

### 2.5. Mechanistic study on catecholaminergic and serotonergic systems

The dose of APG (50 mg/kg) at which the best antidepressant-like effect was observed in TST and MFST was selected in this mechanistic study. Intraperitoneal administration of AMPT, 100 mg/kg, was done 4 h before giving APG or saline (control) and 60 min later, they were evaluated in TST (Cryan et al., 2002; Can et al., 2013). In other experimental groups, phentolamine (5 mg/kg, i.p.) (Kwon et al., 2010), propranolol (2 mg/kg, i.p.) (Schreiber et al., 2000), SCH 23390 (0.05 mg/kg, s.c.) (Yalcin et al., 2007), and sulpiride (50 mg/kg, i.p.) (Bradford et al., 2010), were given 15 min before administering saline or APG and TST was done 60 min later (Gavello-Baudy et al., 2008). The experiment was also repeated with PCPA (inhibitor of tryptophan hydroxylase) and ondansetron to determine the role of the serotonergic system (5-HT3 receptor antagonist). In PCPA group, mice were given PCPA (100 mg/kg) for four days and subsequently APG or saline were administered and 60 min later, TST was done (Can et al., 2017;

Kesim et al., 2005). In the other group, 0.3 mg/kg of ondansetron was given to mice 15 min before APG administration and 60 min later TST was performed.

## 2.6. Statistical analysis

In each group, six animals were used to collect data for the analysis. For statistical analysis, GraphPad Prism was employed. A one-way analysis of variance (ANOVA) and Tukey's test were used to assess the data from the MFST and TST. ANOVA and Bonferroni post hoc test was employed to examine the outcomes of the mechanistic experiments. When the P-value between the groups was less than 0.05, the differences were considered significant, and the data in the figures were expressed as mean  $\pm$  S.E.M.

## 3. Results

### 3.1. Antidepressant test using TST

As indicated in Fig. 1, significant ( $P < 0.001$ ) reduction in the immobility time was recorded in animals with fluoxetine and APG treatment. Among APG doses, when compared to control group, high dose of APG (50 mg/kg) ( $P < 0.001$ ) was able to shorten the immobility phase significantly more than the low dose of APG (25 mg/kg) ( $P < 0.05$ ).

### 3.2. Antidepressant test using MFST

In groups of animals treated with APG and fluoxetine, immobility time was significantly ( $P < 0.001$ ) increased when compared to control group (Fig. 2-A). On the other hand, only non-significant decrease was noted in group of animals that received low dose of APG. Significant ( $P < 0.001$ ) increase in the swimming (Fig. 2-B) and climbing (Fig. 2-C) time were observed in animals that were administered with high dose of APG and fluoxetine when compared to control animals.

### 3.3. Catecholaminergic antagonism on antidepressant like activity of APG

As depicted in Fig. 3, pre-treatment of animals with AMPT, phentolamine, propranolol, SCH, and sulpiride prior to the admin-

istration of APG caused a significantly ( $P < 0.001$ ) enhanced period of immobility in comparison to a group that received only APG in tail suspension tests. Out of all catecholaminergic antagonists, propranolol, a non selective beta blocker, showed decreased duration of immobilization, when compared to other compounds. Nevertheless, total period of immobilisation by propranolol was still significantly ( $P < 0.05$ ) high when compared to APG treated animals.

### 3.4. Serotonergic antagonism on antidepressant like activity of APG

The administration of APG resulted in significant fall in the period of immobility in mice in comparison to normal vehicle treated animals (Fig. 4) in tail suspension tests. Pretreatment of mice with PCPA exhibited a significantly increased ( $P < 0.001$ ) immobility period than those animals that received only APG. The results of Post hoc analysis demonstrated significant decrease in antidepressant like effect of APG in the form of recovering the immobility time in animals that were administered ondansetron prior to APG treatment.

## 4. Discussion

This study looked at the antidepressant-like effect of apigenin (APG) at 25 and 50 mg/kg using established animal experimental models. TST was also used to determine the possible antidepressant effect of APG on catecholaminergic and serotonergic systems using the most effective dose of APG (50 mg/kg).

Intraperitoneal administration of APG at 25 (low dose) and 50 mg/kg (high dose) caused a substantial fall in the duration of immobility in TST, however, high dose was more effective than low dose. The role of APG in reducing the immobility is attributed to enhanced spontaneous motor activity which is normally seen at higher concentration of APG (Nakazawa et al., 2003a; 2003b). A study done by Zanoli et al. (2000) has demonstrated a reduction in the motor activity by APG even at 25 mg/kg, however, we observed a greater reduction in the immobility phase at high dose (50 mg/kg).

We used established animal experimental models for the study. Both MFST and TST are commonly employed animal tests for checking antidepressant-like actions (Weng et al., 2016; Can et al., 2012). The MFST measures not just the animals' passive immobility, but also their actions like swimming and climbing

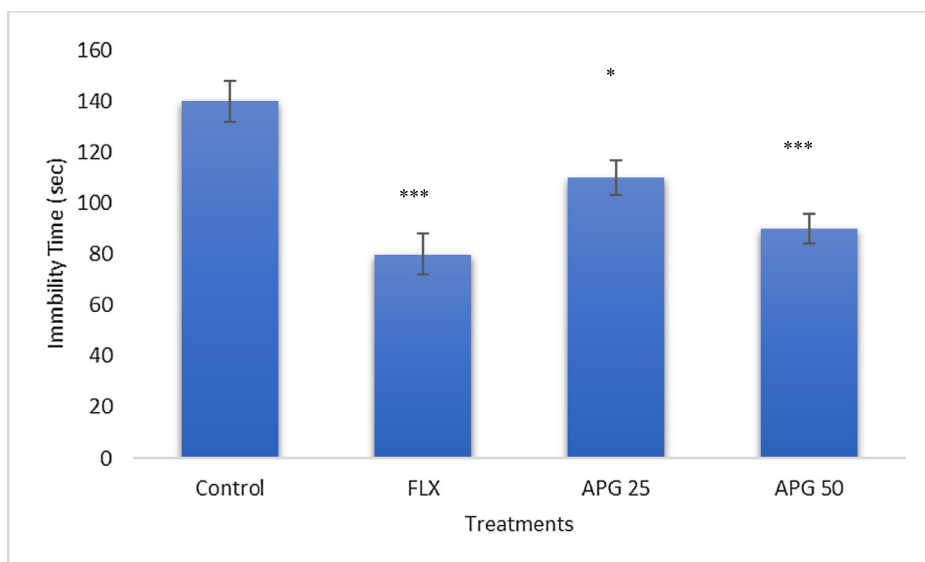
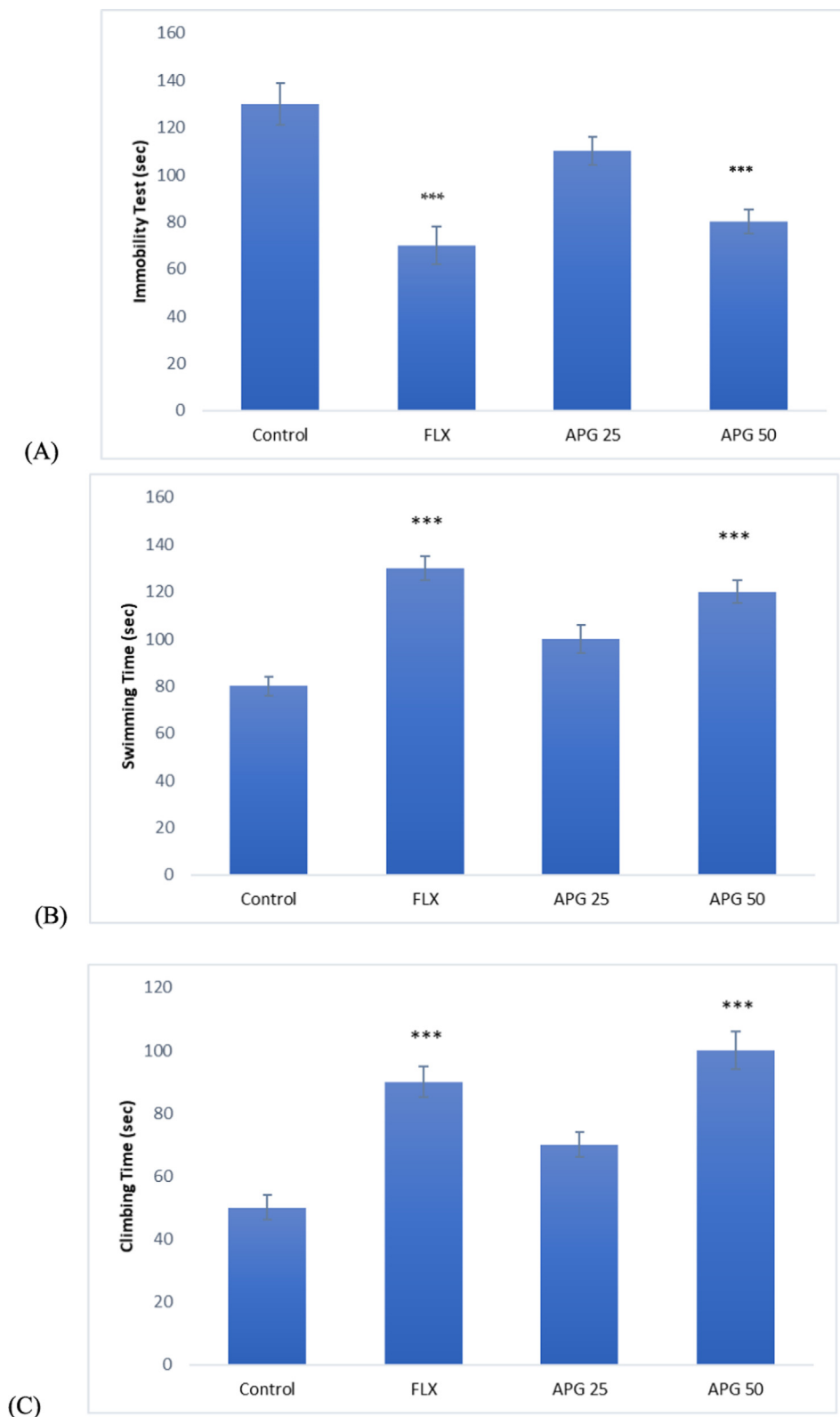


Fig. 1. Antidepressant-like effect in TST. Values are expressed as mean  $\pm$  SEM for 6 animals; APG 25: Apigenin 25 mg/kg; APG 50: Apigenin 50 mg/kg; FLX: fluoxetine; \*\*\* $p < 0.001$  and \* $p < 0.05$  Vs control.

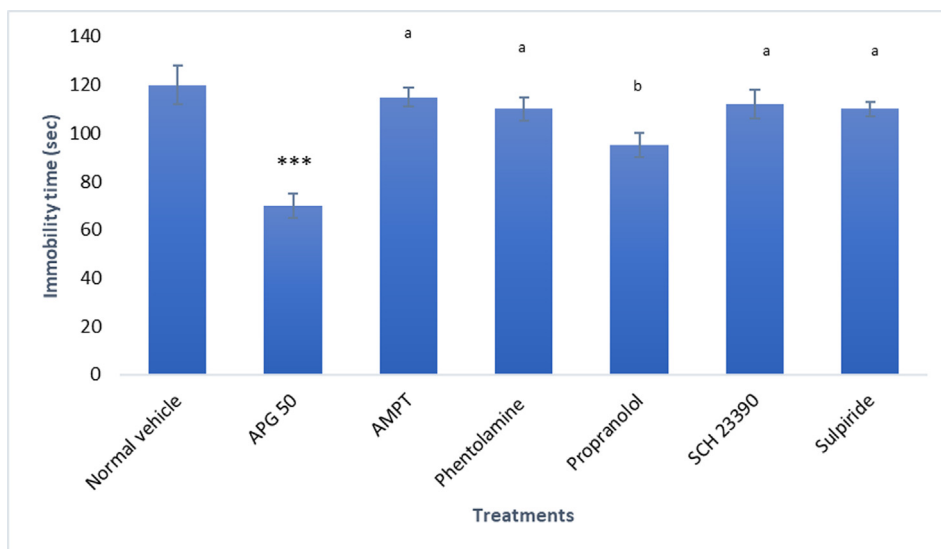


**Fig. 2. Antidepressant like effect using modified forced swimming test.** (A): Period of immobility (sec); (B): Swimming time (sec); (C): Climbing time (sec); data are given as mean ± SEM for 6 animals; APG 25: Apigenin 25 mg/kg; APG 50: Apigenin 50 mg/kg. FLX: fluoxetine 30 mg/kg; \*\*\*p < 0.001 Vs control.

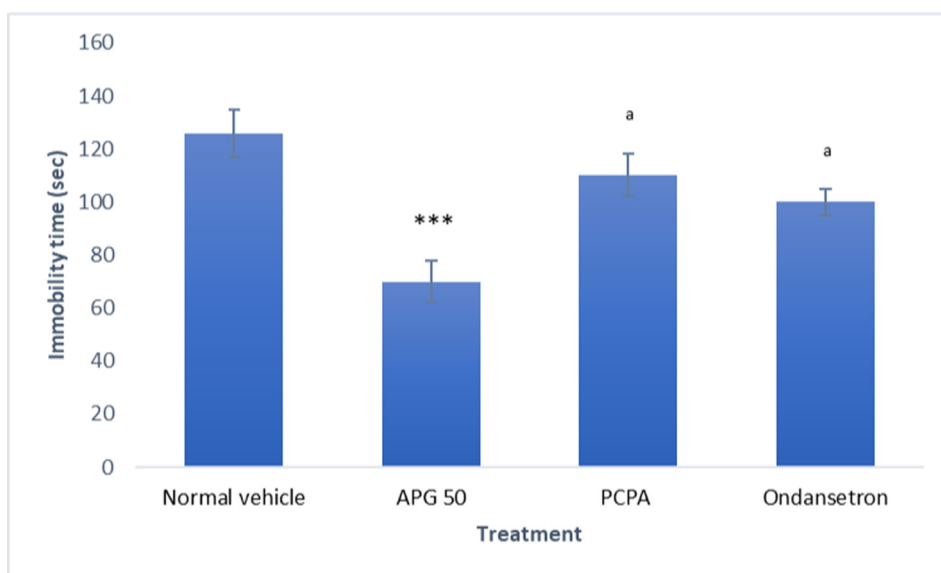
(Slattery and Cryan, 2012; Cryan et al., 2002). In TST, animals were hanged with their tail to induce the immobility (Slattery et al., 2012).

The outcome of the MFST demonstrated the potential benefit of APG at high dose. It caused significant reduction in the period of

immobilization in mice. As expected, fluoxetine (30 mg/kg) reduced the length of immobility behavior. In addition, APG therapy lengthened the duration of swimming and climbing activities. In MFST, the reduction of the immobility time after the administration of APG and the consequent increase in the duration of swimming



**Fig. 3. Immobility time due to pre-treatment of Catecholaminergic antagonist.** Values are expressed as mean  $\pm$  SEM for 6 animals; APG 50: Apigenin 50 mg/kg; \*\*\* $p$  < 0.001 Vs normal vehicle; <sup>b</sup> $p$  < 0.001 and <sup>a</sup> $p$  < 0.05 Vs to normal APG 50.



**Fig. 4. Immobility time due to pre-treatment of serotonergic antagonist.** Values are expressed as mean  $\pm$  SEM for 6 animals; APG 50: Apigenin 50 mg/kg; \*\*\* $p$  < 0.001 Vs normal vehicle; <sup>a</sup> $p$  < 0.001 and <sup>a</sup> $p$  < 0.05 Vs normal APG 50.

and climbing demonstrated the antidepressant effect of APG. In TST, as with MFST, APG administration decreases the period of immobility in mice. The result of TST complements the results obtained from MFST in which APG at high dose produced a remarkable decrease in the period of immobilization. Additionally, the results of these two tests confirm the findings of the earlier report that demonstrated antidepressant effects of APG (Cryan et al., 2005).

After testing the potential anti-depressant activity of APG, we conducted several studies of the mechanism to determine the possible contribution of the monoaminergic system for its possible therapeutic benefits. The TST was selected for this part of the study as this test is reported to be more drug sensitive without any risk of hypothermia and rapid spontaneous recovery at the end of the trials (Porsolt et al., 2001).

The participation of operating systems of catecholamines that are possibly responsible for antidepressant activity of APG was determined using several catecholaminergic antagonists. The role

of noradrenaline and dopamine in APG mediated antidepressant like activity was determined using  $\alpha$ -methyl-*para*-tyrosine methyl ester (AMPT). Administration of AMPT is known to increase the brain norepinephrine and dopamine by approximately 50% without any effect on serotonin (Weng et al., 2016; Cryan et al., 2005). Our study results show successful reversal of APG mediated antidepressant potential by prior administration of AMPT. Further, since both adrenergic and dopaminergic systems may take part in the antidepressant action of several agents (Widerlöv et al., 1978), we carried out studies involving number of dopamine receptor antagonists.

Pretreatment of animals with AMPT prior to APG decreases the anti-immobility effect of APG in TST. As reported earlier (Mayorga et al., 2001), both adrenergic and dopaminergic systems could be responsible for possible anti-depressant potentials, therefore we carried out further experiments using specific receptor antagonists. Prior injection of phentolamine reversed the antidepressant action of APG more strongly than propranolol indicating the bigger role of

alpha adrenoceptor than the beta adrenoceptor in modulating the anti-depressant potential of APG. The dopaminergic receptor participation in the APG mediated antidepressant effect was confirmed by prior administration of SCH 23390 and sulpride. Both compounds significantly able to reverse the shortened immobility time as a determinant of antidepressant effect of APG. Taken together, increased catecholamine levels in the synapse were found play a central role in the possible anti-depressant activity of APG, particularly, the receptors of dopaminergic (D1, D2, D3) and alpha-adrenergic systems.

The role of serotonergic receptors in the possible antidepressant potential of APG was determined by prior administration of PCPA and ondansetron. P-chlorophenylalanine methyl ester (PCPA) was administered for four days to end the biosynthesis of serotonin as an inhibitor for tryptophan hydroxylase and therefore decreases the reserves of serotonin by around 60–90% (Pytko et al., 2016; Koe and Weissman, 1966). We observed a substantial recovery in the period of immobilization of mice that were given PCPA prior to the administration of APG. Also, we found a remarkable increase in the period of immobilization of the animals that were given ondansetron (5-HT<sub>3</sub> receptor antagonist) signifying the possible role of 5-HT<sub>3</sub> receptor in potential anti-depressant activity of APG. Earlier studies have demonstrated varying role of 5-HT<sub>3</sub> receptors on the regulation of depression. 5-HT<sub>3</sub> receptor agonists and antagonists present different affinity and efficacy depending on the structure of 5-HT<sub>3</sub> receptors, i.e., heteromeric or homomeric (Hapfelmeier et al., 2003; Stewart et al., 2003). 5-HT<sub>3</sub> receptor agonists seem to counteract the effects of antidepressants in non-clinical models, whereas 5-HT<sub>3</sub> receptor antagonists, such as ondansetron, present antidepressant-like activities. The antidepressant effect of ondansetron was reported to be dose dependent. When compared to other sertrons, ondansetron has decreased inhibition of internalization of 5HT<sub>3</sub> receptors and therefore their antidepressant effect is not so dominant like palanosetran (Rojas et al., 2010). Another report suggested a depression inducing property of ondansetran when used as anti-emetic in doxorubicin induced nausea (Blaine, 1997). Therefore, it is possible that the ondansetran induced short lived depression was successfully reversed by APG possibly through 5HT<sub>3</sub> receptor mediation. However, further molecular evaluation are required to confirm the findings of this study. It is also recommended that until further information about the anti-depressant effects of APG becomes available, caution should be exercised in their usage.

## 5. Conclusion

To conclude, APG seems to have two mechanisms for exhibiting its antidepressant effect that involve not only serotonergic but also catecholaminergic transmission. Additionally, alpha adrenergic receptor, 5 HT<sub>3</sub>, D1, D2 and D3 receptors shown to be participating in the pharmacological effect. However, there is a need to explore other pharmacological mechanisms that include GABAergic, glutaminergic and several other mechanisms that may play a role in APG mediated antidepressant like action.

## Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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