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ANTIDEPRESSANT ACTIVITY OF A POLYHERBAL FORMULATION R013, IN EXPERIMENTAL ANIMAL MODEL

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ABSTRACT

RO13, a poly herbal formulation is indicated for the management of depressive state but there was an absence of substantial pharmacological data. Hence, the present study was attempted to prove the antidepressant property of RO13, in conventional animal models of depression. Albino mice were grouped into four, each group with 6 animals and injected with Normal Saline (control), Imipramine-10mg/kg (Standard drug), RO13 aqueous extract 200mg/kg and 400mg/kg (test drug) orally. The animal groups were subjected to Tail Suspension Test and Force swim test. The results showed that in both Tail suspension test and Force swim test, the aqueous extract of RO13 at dose of 200mg/kg and 400 mg/kg significantly reduced immobility time ($p<0.01$) in acute studies and further reduced in chronic studies. However, the effect was increased in swimming ($P<0.01$) and insignificant change in climbing in acute studies and increased in duration of swimming and climbing ($P<0.05$ &$P<0.01$) in chronic studies in FST. The aqueous extract of RO13 possesses dose dependent antidepressant effect. Current studies confirm the claimed antidepressant activity in selected doses (200mg/kg and 400mg/kg).

Key words: Antidepressant, Polyherbal formulation, RO13, Tail suspension test, Forced swim test.

INTRODUCTION

Mental depression is one of the common chronic illnesses, which is defined as disorders of mood rather than conflicts of thought or cognition; it may range from a very mild condition, bordering on normality to severe (psychotic) depression accompanied by hallucinations and delusions. Although the mechanism involving depression has not been clearly elucidated, the main trigger is known to be exposure to unceasing stress (Gopalakrishna *et al.*, 2010).

According to the World Health report, approximately 450 million people suffer from a mental or behavioural disorder. It was shown that depression is the fourth primary cause of disability worldwide exceed by lower respiratory infections, prenatal conditions and HIV/AIDS (World Health Organization, 2001). The disorder was characterized by apathy, loss of energy, retardation of thinking and activity, as well as profound feelings of gloominess, despair and suicidal ideation. In spite of the availability of antidepressant drugs like tricyclic antidepressants, selective reversible inhibitors of monoamine oxidase –A (MAO – A), selective serotonin reuptake inhibitors (SSRIs) and selective nor-adrenaline reuptake inhibitors (SNRIs), depression continue to be a major medical problem (Yu Z *et al.*, 2002).

Various plants are being used in complementary and alternative medicines for management of stress and mood disorders, because of their minimum toxicity and more effectiveness (Santosh P *et al.*, 2011). Herbal medicines are less toxic and less costly when compared to the synthetic drugs (Chowdhury *et al.*, 2009). According to practitioners of traditional medicine, a combination of herbs exhibits augmented therapeutic efficacy than a single herb (Toews *et al.*, 2005). So in the present study an attempt has been made to identify the scientific proof for claiming the antidepressant activity of the marketed poly herbal formulation RO13.

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The Poly herbal formulation RO13 contains: *Eclipta alba, Glycyrrhiza glabra, Hemidesmus indicus, Hibiscus rosa-sinensis, Nelumbo nucifera, Quercus infectoria, Rosa damascena, Terminalia chebula, Zingiber officinalis.* Among these, *Zingiber officinalis, Terminalia chebula ,Nelumbo nucifera, Eclipta alba, Glycyrrhiza glabra , Rosa damascena* had already been shown to exhibit antidepressant activity in experimental models in previous studies.

MATERIALS AND METHODS

Chemicals and reagents

RO13, a polyherbal formulation was gifted by Rumi Herbal Research Institute Private Limited, Chennai. Standard Imipramine was obtained from Torrent pharmaceutical Ltd. Gangtok, Sikkim.

Method of preparation of RO13

5 g of RO13 was added to 150 ml of boiling water and boiling continued for 2 min. The decoction was cooled, filtered and the filtrate 35 ml is considered to represent 5 g of RO13. This was orally administered to Albino mice using oral hypodermic needle. (Sudhakar *et al*., 2010).

Preliminary phytochemical group test

The preliminary phytochemical group test of the aqueous extract of RO13 was performed by the standard methods to see the various chemical constituents. (Tyler *et al*., 1993, Plummer DI 2002)

Animals

Albino mice (20 - 30 g) were collected from Sri Venkateshwara Enterprises No.4304, 13th main, 1st cross, Subramanyamnagar, Bangalore-560021. Animals were housed in groups of four (each carry 6 animals) at an ambient temperature of 25 ±1^{o}C. Animals had free access to food and water. Animals were deprived of food but not water before 4 hr of the experiment. Ethical committee clearance was obtained for the animal use from IAE (Institutional Animal Ethics Committee) of CPCSEA (Ref. No.461/01/C/CPCSEA).

Acute toxicity studies

The acute toxicity of RO13 was determined as per the OECD guideline no. 423 (Acute toxic class method, 2002). It was observed that the rats were not mortal even at 2000 mg kg-1 dose of RO13. Hence, 1/5th (400mg/kg) and 1/10th (200 mg/ kg) of MCPB were selected as high dose and low dose respectively for this study.

Experimental design

On the day of the experiment, the animals were divided randomly into control and experimental groups (n=6). Group 1 received the normal saline, 1% gum acacia (10ml/kg) and served as the control group, group 2 received the standard drug imipramine (10mg/kg) *per orally*. Groups 3 and 4 received the test drug (aqueous extract of RO13) in doses of 200 and 400 mg/kg. Drugs/vehicle was administered to the animals 60 minutes prior to the behavioural evaluation in acute study. For chronic study, a new set of animals were used. They were grouped as in acute study and were administered the drugs/vehicle for a period of 10 days. Behavioural evaluation was carried out 60 minutes post drug/vehicle administration on 10th day. The antidepressant activity of the test drug was evaluated using the following experimental models of depression TST and FST (Toews *et al.,* 2005).

Tail suspension test (TST)

Steru *et al*., (1985) method was used to perform the TST. The animals were hung by the tail on a plastic string 75 cm above the surface with the help of an adhesive tape. The duration of immobility was observed for a period of 8 minutes. The duration of immobility was recorded during the last 6 minutes of the observation period. Mice were considered to be immobile only when they were completely motionless while they were hung up passively.

Forced Swim Test (FST)

For FST Porsolt *et al*., (1977) method was used. Each animal was positioned individually in a 5 litre glass beakers, filled with water up to a height of 15 cm and were observed for duration of 6 minutes. The duration of immobility was recorded during the last 4 minutes of the observation period. The mouse was considered immobile when it floated motionlessly or made only those moments necessary to keep its head above the water surface. The water was changed after every test.

Statistical Analysis

Data are expressed as Mean ± SEM. The results were subjected to one-way analysis of variance (ANOVA), followed by Dunnet's multiple comparison test to compare the treatment groups with control group.

RESULTS

The preliminary phytochemical tests of RO13 showed the presence of steroids, triterpenoids, alkaloids, flavonoids, tannins, amino acids, reducing sugar and saponins.

Tail suspension test

Results are given in table 1. In acute studies, the aqueous extract of RO13 significantly reduced the duration of immobility at dose of 200mg/kg ($p<0.05$) and 400 mg/kg ($p<0.01$) and further reduction of immobility

was seen in chronic studies after 10 days of daily treatment of 200mg/kg ($p<0.05$) and 400 mg/kg ($p<0.01$) when compared with the control.

Force suspension test

In FST, the extract of RO13 significantly decrease the immobility time at dose of 200mg/kg ($P<0.05$) and 400mg/kg ($P<0.01$) Table 2. There is a prominent increased in the duration of swimming ($P<0.01$) in all the groups as compared to the control but there was an insignificant change in the duration of climbing as compared to control in acute studies. After chronic treatment, there was further significant reduction in duration of immobility and increased in duration of swimming and climbing ($P<0.05$ & $P<0.01$).

Table 1. Duration of immobility in Tail suspension Test

Groups	Duration of immobility(sec)	
	Acute studies	Chronic studies
Group 1 (normal saline)	222.50±03.56	217.16±02.63
Group 2 (Imipramine 10mg/kg)	139.83±02.02**	122.66±03.28**
Group 3 (RO13 aqueous extract, 200mg/kg)	165.33±02.99*	150.50±02.32*
Group 4 (RO13 aqueous extract, 400mg/kg)	149.33±01.90**	130.50±2.04**

Value represented mean ± S.E.M (n=6) * p<0.05 vs. control, ** p<0.01 vs. control (group 1). One-way ANOVA followed by Dunnet's Test.

Table 2. Duration of immobility in FST

Groups	Duration of immobility(sec)	
	Acute studies	Chronic studies
Group 1 (normal saline)	192.16±2.77	165.33±3.56
Group 2 (Imipramine 10mg/kg)	91.5±3.19**	82.66±1.69**
Group 3 (RO13 aqueous extract, 200mg/kg)	121.50±4.35**	114±5.29**
Group 4 (RO13 aqueous extract, 400mg/kg)	95.33±2.45**	83.66±4.19**

Value represented mean ± S.E.M (n=6) * p<0.05 vs. control, ** p<0.01 vs. control (group 1). One-way ANOVA followed by Dunnet's Test.

Table 3. Duration of swimming in FST

Groups	Mean Duration of Swimming in sec	
	Acute studies	Chronic studies
Group 1 (normal saline)	47.83±5.66	68.16±3.01
Group 2 (Imipramine 10mg/kg)	165.33±3.89**	189.14±4.25**
Group 3 (RO13 aqueous extract, 200mg/kg)	108.32±2.56**	159±1.08**
Group 4 (RO13 aqueous extract, 400mg/kg)	160.11±1.66**	187.01±3.18**

Value represented mean ± S.E.M (n=6) ** p<0.01 vs. control. One-way ANOVA followed by Dunnet's Test.

Table 4. Duration of climbing FST

Groups	Mean Duration of climbing in sec	
	Acute studies	Chronic studies
Group 1 (normal saline)	20.50±15.04	8.83±3.27
Group 2 (Imipramine 10mg/kg)	3.50±1.20^{ns}	0.50±0.34^{ns}
Group 3 (RO13 aqueous extract, 200mg/kg)	5.00±2.40^{ns}	0.833±0.54*****
Group 4 (RO13 aqueous extract, 400mg/kg)	6.16±1.20^{ns}	2.50±1.17******

Value represented mean ± S.E.M (n=6) ** p<0.01 vs. control. One-way ANOVA followed by Dunnet's Test.

DISCUSSION AND CONCLUSION

The anti-depressant-like activity of polyherbal drug, RO13 was expressed by a decreased duration of immobility in FST and TST. Both these models of depression are widely used to screen new chemical (Steru *et al.*, 1985; Porsolt *et al.*, 1977). The studies were done in 2 stages – acute study and chronic study that extend to 10 days of daily administration. Since it is well known fact that treatment for depression needs administration of antidepressant drugs for 2-6 weeks before clinical efficacy is observed (Malberg *et al.*, 2005). In the present study, we selected behavioural despair models, namely tail suspension test and forced swim test in mice to evaluate the claimed antidepressant effect possibly

involving noradrenergic and serotoninergic mechanisms. Acute administration of most antidepressants decreases immobility (Bhattacharya *et al*., 1999).

We observed that following in acute studies, the aqueous extract of RO13 proved *significant* (compared to saline group), a dose dependent reduction in duration of immobility. The effect of the aqueous extract at the dosage of 400mg/kg is nearly similar to that of the Imipramine (10mg/kg). Further, the duration of immobility was reduced after 10 days of daily administration (Chronic studies). The effect continues to be statistically significant when compared to vehicle treated animals.FST was originally interpreted by Porsolt *et al* as *"behavioural despair*" such that the animal has lost the motivation to perform escape oriented behaviour (Porsolt *et al*., 1977). Activity and immobility in the FST can less anthromorphically be interpreted as representing alternately active and passive behavioural reactivity to stress (Holmes *et al*., 2003). Antidepressants, of all major classes reduce immobility and increase active behaviours in forced swim test.

Qun Xu *et al*., is of the opinion that the traditional immobility behaviours may give rise to a false positive effect in mouse forced test and suggests procedure that involved detailed behavioural scoring, which adds evaluation of swimming and climbing to the rational immobility floating measures (Qun Xu *et al*., 2008). Furthermore, the scoring behaviours of swimming and climbing give additional information about the mechanism of the action that mediates the antidepressant like effects. Swimming is mediated by serotoninergic neurotransmission and climbing is mediated by norepinephrenergic neurotransmission (Detke *et al*., 1995; Lucki *et a.l* 1997). Based on this method, drugs that inhibit norepinephrine reuptake, decrease immobility time without any significant effect on swimming time. Drugs that selectively inhibit serotonin reuptake decrease immobility and increase swimming time (Detke *et al*.,, 1995).

In Force swim test, the aqueous extract of RO13 at dose of 200mg/kg and 400 mg/kg significantly increased in swimming and insignificant change in climbing in acute studies and increased in duration of swimming and climbing in chronic studies. However, the effects on climbing behavioural aspects were not clear in this study.

The most prevalent theory for the pathogenesis of depression is "Monoamine Hypothesis". Functional deficiency of central monoamines such as noradrenaline, 5-hydroxytryptamine and dopamine are responsible for the symptoms of depression (Schildkraut 1965). Many currently used antidepressants act by increasing the concentration of these neurotransmitters in the brain (Malberg *et al*.,, 2005). Therefore, the Antidepressant-like activity of RO13 might be due its modulatory effect on central monoamines. Data obtained in our preclinical study allows us to propose this RO13, polyherbal formulation as an excellent candidate for antidepressant activity.

Finally, we can conclude that RO13 showed a comparable effect as that of Imipramin in both acute and chronic studies but as RO13 is a polyherbal formulation it is expected to have low side effects. Hence, In the near future Imipramin can be replaced by RO13 to get rid of the side effects of other antidepressant drugs.

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