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Original Article

Anxiolytic Antidepressant and Anti-Inflammatory Activity of Ethanolic Extract of Urena Lobata Leaf

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ABSTRACT

The effect of urena lobata extract was studied for the anxiolytic, antidepressant and anti-inflammatory activities. Swiss albino mice and male sprague dawley rats were used for the studies. All the animals were grouped into different groups and taken for the studies. Anxiolytic activity was studied using elevated plus maze test, dark light transition model and for antidepressant activity tail suspension method, forced swim test were employed and for anti-inflammatory activity carrageenan induced rat paw edema model was used. Anxiolytic activity was observed with urena lobata leaf extarct at 500 mg/kg which shows clearly anxiolytic effects similar to the standard drug diazepam. Urena lobata leaf extract at both 250mg/kg and 500 mg/kg significantly increase of time spent in light area, latency to dark chamber and no. Of tunnel crossingseven the same effect effective in forced-swimming test. Effect of u.l on tail suspension method was studied and imipramine was used as a standard drug, *urena lobata* with 250mg/kg and 500mg/kg doses were studied for the antidepressant activity. The leaf extract of *urena lobata* at a dose of 500 mg/kg significantly inhibited carrageenan induced edema ($p<0.05$) after 60 min. These studies lead to the conclusion that urena lobata leaf extract could be used for the treatment of anxiety, depression and inflammatory, however elucidation of exact mechanism of action and beneficial effects of this extract need further investigation.

Key words: *urena lobata* leaf extract, swiss albino rats, tail suspension method, forced swim test, carrageenan, swiss albino mice, light-dark transition, elevated plus maze.

1. INTRODUCTION

The usage of herbal medicines was recognized by WHO. According to WHO three-quarters of the world's population are using herbal medicines to treat their diseases. WHO has recently defined traditional medicine (including herbal drugs) as comprising therapeutic practices that have been in existence, almost for several hundred years. The traditional preparations comprise medicinal plants, minerals, organic matter, etc. herbal dugs constitute only those traditional

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medicines, which are primarily use medicinal plants preparations for therapy [1]. Depression is nothing but the mood disorder. 25% of women and 12% of men are suffering due to depression [2]. To treat the depression anti depressant drugs are used. They act on the central monoaminergic systems, 5-HT and nor-adrenergic synaptic neurotransmissions mainly. Selective serotonin reuptake inhibitors and noradrenaline reuptake inhibitors are also shows the best results in treating mental disorders [3]. The major cause for the neurodegenerative diseases and mental disorders is stress [4]. Restraint stress for 120 min has been reported to enhance depression-like behavior in mice [5]. Acute exposure to 2 h of restraint stress exhibited a decrease in the concentration of 5-HT and its metabolite – 5-hydroxyindoleacetic acid, in the hippocampus, leading to stress-induced behavioral depression [6]. NO is a short lived, lipophilic molecule generated from L-arginine by nitric oxide synthase. There are three NOS isoforms – iNOS, nNOS and eNOS [5]. NO production is increased in depression [7]. A differential role is played by neuronal and in-ducible isoforms of NOS in depression in mice under unstressed and stressed conditions. Acute restraint stress has been observed to significantly increase expression of iNOS and plasma nitrite levels in rodents [8-10]. Aminoguanidine, an inhibitor of inducible NOS, reversed stress-induced depression-like behavior in rats. The antidepressant-like activity of 7-nitroindazole and 1-(2 trifluoromethylphenyl) imidazole, inhibitors of neuronal NOS, have been reported in unstressed mice [11 – 13]. Anxiety is nothing but the fear related to specific situations it occurs normally in all kinds of people. A recent study states that frequency of occurring anxiety in life time is 28.8% [14]. It also leads to many psychiatric and mental disorders. The most effective treatment is anxiolytic drugs. Most of them are untreated due to side effects causing by these drugs. So, herbal medicines are proposed to be used to avoid the side effects [15]. Inflammation is any damage or injury occurs to the tissue caused by bacteria, trauma, chemicals, heat, or any other phenomenon and the responses lead to swelling, redness, often painful is seen. Cardinal signs of inflammation are rubor, redness; calor, heat (or warmth); tumor, swelling; and dolor, pain; a fifth sign, functiolaesa [16]. The entire complex of tissue changes is called inflammation. The destruction or removal of the injurious material and the responses that lead to repair and healing [17]. *Urena lobata* is an annual, variable, erect, ascendant under shrub and measuring up to 0.5 to 2.5 meters tall. It is widely distributed as a weed in the tropics of both hemispheres including Southeast Asia [18]. The traditional uses of the plant were found to be diuretic, febrifuge and on the treatment of rheumatism. It is also used for gonorrhea, wounds toothache and also used for food for animals as well as humans previous research by other workers on the aerial part of the plant yield mangeferin and quercetin. Triglycerides were isolated from the plant and imperatorin and a furocoumarin was isolated from roots of Urena lobata [19-20]. Therefore, the present study was designed to explore antidepressant activity, anxiolytic and anti inflammatory activity of Urena lobata leaves. The plants are rich in alkaloids and saponins which are known to have antimicrobial activity as well as other physiological activity. Flavonoids are known for their vast role in biological activities which include protection against allergies, viruses and tumors, ulcers, inflammation and platelets aggregation. These flavonoids are potent water-soluble super antioxidants and free radical scavengers which provide protection against oxidative cell damage. They also provide antioxidative properties against some certain forms of cancer and protect against all stages of carcinogenesis [21 – 22].

2. MATERIALS AND METHODS

ANIMALS

Male Sprague Dawley Rats (200 – 250 gm), Male swiss Albino mice (22 – 25 gm) were employed in study. The rats will have free access to standard pellet feed and water ad libitum and are to be housed under controlled temperature 25^{o}C ± 2^{o}and relative humidity 44-56%. A light dark cycle of 12:12h respectively used. Nutritious animal feed was purchased from Royan biotechnologies pvt. Ltd, Hyderabad. Animal **e**xperiments were carried out after approval from the IAEC constituted by CPCSEA (IAEC Approval no: 005/IAEC/NCPA/M.Pharm/2014-2015)

PREPARATION OF PLANT EXTRACTION: The plant material was made into small pieces, dried under shade and powered. The coarse powder was extracted with ethanol using cold percolation method. This is similar to the traditional method of extraction used by herbalists throughout the India. A known amount of the dried material (5gm/ 50 ml) was soaked in ethanol and kept for continuous shaking nearly 48hrs using percolator. This was followed by filtration by using vacuum filtration and evaporation of excess solvent without applying heat. The obtained dried extract was stored at - 4°C (Kokate CK) and obtained extract yield after dried was approximately 2.5 gms for 25 gms of bark powder.

ACUTE ORAL TOXICITY STUDIES-OECD 423:

The animals were randomly selected, marked to permit individual identification, and kept in their cages for at least 5 days prior to dosing for acclimatization to the laboratory conditions. The animals had been fasted overnight during period of drug administration with complete access to water all the time. Following the period of fasting, the animals were weighed and the test substance was administered. Three animals were used for each step. The dose levels selected were 5, 50, 300 and 2000mg/kg body weight. The time interval between treatment groups was determined by the onset, duration, and severity of toxic signs. Treatment of animals at the next dose, delayed until confidence of survival of the previously dosed animals. Animals were observed individually after dosing at first 30 minutes, periodically during the first 24 hours, with special attention given during

the first 4 hours, and daily All observations were systematically recorded with individual records being maintained for each animal. All the animals dosed with different doses did not show any kind of abnormal clinical signs. Hence the extract was considered safe to use for the animal experiments.

ELEVATED PLUS MAZE TEST:

The animals were divided in to four groups, with each group consisting of 5 male mice. First group receives normal saline, second group received diazepam (1mg/kg), third consists of standard drug piracetam, fourth group and fifth one receives plant extract 250 mg/kg and 500 mg/kg respectively. The plus – maze consists of two open arms and two closed arms (50 x 10 x 40 cm each) elevated to a height of 50 cm. Thirty minutes post treatment, each mouse was placed in turn in the centre of the maze facing one of the closed arm. The cumulative times spent by each mouse in the open and closed arms of the maze will be recorded for 5 to 7 min [23 – 24].

LIGHT-DARK TRANSITION

Swiss albino mice (20-25 g) of either sex will be divided into 4 groups of four mice in each will be fasted overnight prior to the test but water will be supplied ad libitum. Group 1 received normal saline, Group 2 received std drug, Group 3 received 250 mg/kg plant extract, and group 4 received 500 mg/kg plant extract. At the starting of the experiment, the mouse will be placed in the illuminated part of the cage. The parameters such as Latency to the first crossing into the dark compartment, number of crossings between the light and dark areas, total time spent in the illuminated part of the cage was recorded during the test session of 5 min: [25 -26].

FORCED SWIM TEST:

Male Sprague Dawley Rats (200 – 250 gm) were used for the experiment. They will be individually forced to swim in an open cylindrical container (diameter 10 cm, height 25 cm), containing 19 cm of water at 25 ± 1º C. All the rats were divided into different groups. The first group (depressed animals) assigned as control received only vehicle (0.9% Normal saline- 10ml/kg, i.p or 20% Tween80, p.o). The second group received standard drug Imipramine (10mg/kg, p.o), the other two groups received 250mg/kg and 500mg/kg of the extract. The total duration of immobility were recored during 10-min period. Each Rat was judged to be immobile when it ceased struggling and remained floating motionless in the water, making only those movements necessary to keep its head above water. A decrease in the duration of immobility is indicative off an antidepressant effect [27].

TAIL SUSPENSION TEST:

Male swiss Albino mice (22 – 25 gm) were used for this study. All the mice were divided into different groups; each group consists of 6 animals. The first group received only vehicle (control), the second group received standard drug Imipramine (20mg/kg, p.o), and other two groups received test drugs 250 mg/kg and 500 mg/kg respectively. Just before 30min prior to testing, the mice were suspended on the edge of a shelf 58 cm above a table top by adhesive tape placed approximately 1 cm from the tip of the tail. The duration of immobility was recorded for period of 5min. Mice was considered immobile when they hang passively and completely motionless for at least 1 min. The percentage of animals showed the passive behavior will be counted and compared with vehicle treated controls, using various doses [28 – 29].

CARRAGEENAN INDUCED PAW EDEMA METHOD:

Sprague Dawley rats (weight 180-200gms and age 2-3 months) of either sex were divided into 5 groups. Acute inflammation were produced by sub plantar injection of 0.1mL of 1% suspension of carrageenan with 2% gum acacia in normal saline, in the left hind paw of rats, one hour after oral administration of extract doses each and Indomethacin 10mg/kg body wt. will be administered by oral route. The paw volume will be measured plethysmometrically (Digital volume meter) at 0, 0.5, 1, 2, and 3hr after the carrageenan injection. The difference between '0' readings and readings after 30, 60, 120 and 180 min respectively will be taken as the volume of edema. Percentage inhibition of edema will be calculated [30].

DRUG TREATMENT

Dose Selection: Doses were selected based on acute toxicity studies.

Animal groups: Experimental animal groups are divided into 6 groups, each group consists of 6 animals (n=6).

Group 1 - Normal control, receives vehicle (oral)

Group 2 - positive control

Group 3 - 1st dose of extract250 mg/kg.

Group 4 - 2nd dose of extract 500 mg/kg

3. RESULTS

Pharmacological Investigations

Table 1: Anti Anxiety Activity By Elevated Plus Maze Model

Group	Treatment	No. of entries / 5min		Time spent (Sec)/5min	
		Open arm	Close arm	Open arm	Close arm
Group I	Control (Vehicle, p.o)	4.50±0.3	7.6±0.8	26.66±0.18	22.01±0.27
Group II	U.L.(250mg/kg)	5.37±0.7	7.45±0.45	28.16±0.37***	10.94±0.36***
Group III	U.L. (500mg/kg	7.2±0.5	5.60±0.7	30.07±0.25***	13.04±0.21***
Group IV	(Diazepam; 10mg/kg	7.66±0.2	4.45±0.6	35.15±0.23***	15.69±0.31***

(Values are in Mean±S.E.M (n=6); ns -Non Significant, *p <0.05, ** p<0.01, *** p<0.001 when compared with Control using One way ANOVA followed by Dunnet's "t" test.).

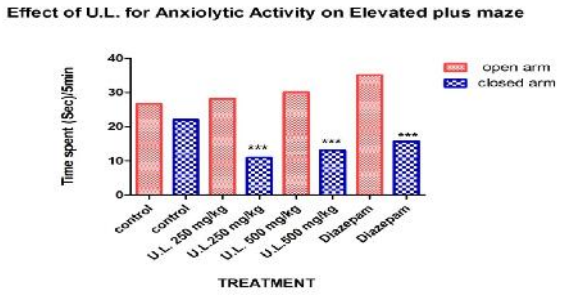

Fig 1: Results were expressed as Mean±SEM . Doses of *U.L.* are 250mg/kg,500 mg/kg.P<0.05,compared with control; Results were analysed by one-way anova using Dunnett's multiple comparison test.**

Table 2: Effect of U.L. On dark-light model for anxiolytic activity.

Groups	Treatment	Time spent in light chamber (Sec) Mean±SEM	Time spent in dark chamber (Sec) Mean±SEM
Group I	Control (Vehicle,10ml/kg, p.o)	28.20±0.86	3.88±0.23
Group II	U.L. (250mg/kg p.o)	15.64±0.27***	7.26±0.25***
Group IV	U.L. (500mg/kg ,p.o	19.26±0.08***	5.70±0.31***
Group V	(Diazepam; 10mg/kg,p.o)	17.88±0.39***	5.40±0.41***

(Values are in Mean±S.E.M (n=6); ns -Non Significant, *p <0.05, ** p<0.01, *** p<0.001 when compared with Control using One way ANOVA followed by Dunnet's "t" test.).

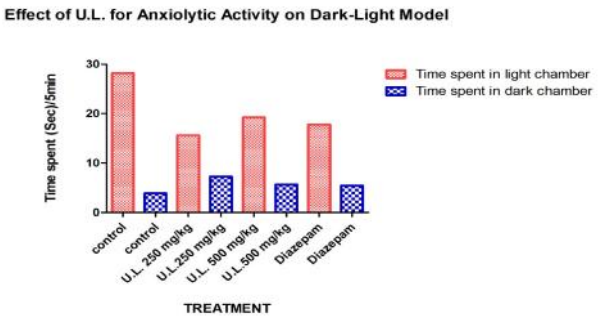

Fig 2: Results were expressed as Mean±SEM . The Doses of *U.L.* are 250mg/kg, 500 mg/kg.P<0.05,compared with control; Results were analysed by one-way anova using Dunnett's multiple comparison test.**

Table 3: Effect of u.l. Extract on forced swim test

Group	Dose (I.P; mg/kg)	Time of immobility in seconds
Control	5ml/kg	149 ± 2.469
Imipramine	30mg/kg	117 ± 2.875**
U.L.	250mg/kg	134 ± 3.276*
U.L.	500mg/kg	125 ± 3.055**

(Values are in Mean±S.E.M (n=6); ns -Non Significant, *p <0.05, ** p<0.01, *** p<0.001 when compared with Control using One way ANOVA followed by Dunnet's "t" test.).

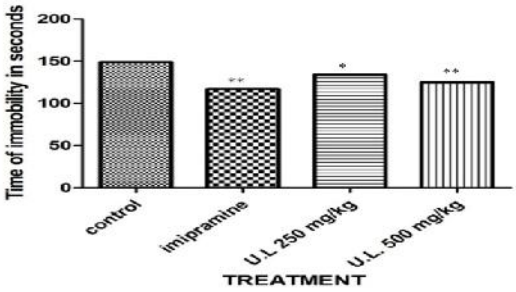

Fig 3: Results were expressed as Mean±SEM . The Doses of *U.L.* are 250mg/kg,500 mg/kg.P<0.05,compared with control; Results were analysed by one-way anova using Dunnett's multiple comparison test.**

Table 4: Effect Of *U.L* On Tail Suspension Method

Group	Dose (i.p; mg/kg)	Time of immobility in seconds
Control	5ml/kg	220 ± 1.56
Imipramine	30mg/kg	127 ± 1.05**
U.L.	250mg/kg	154 ±2.26*
U.L.	500mg/kg	125 ± 2.05**

(Values are in Mean±S.E.M (n=6); ns -Non Significant, *p <0.05, ** p<0.01, *** p<0.001 when compared with Control using One way ANOVA followed by Dunnet's "t" test.).

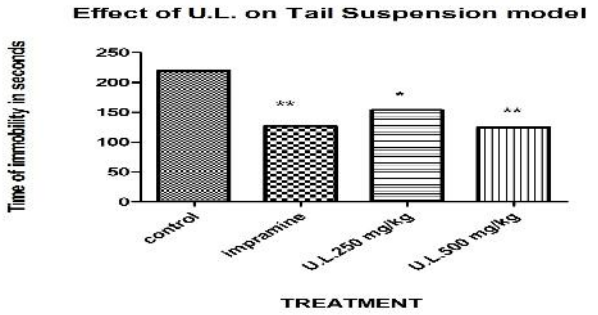

Fig 4: Results were expressed as Mean±SEM . The Doses of *U.L.* are 250mg/kg, 500 mg/kg.P<0.05,compared with control; Results were analysed by one-way anova using Dunnett's multiple comparison test.**

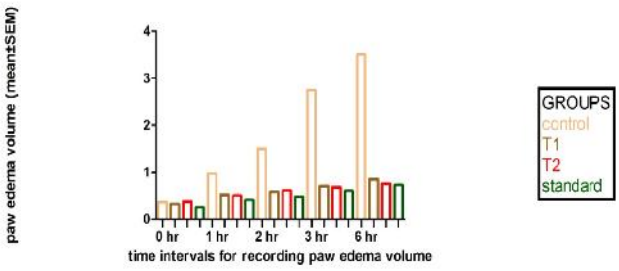

Fig 5: Values are in Mean±S.E.M (n=6); ns -Non Significant, *p <0.05, ** p<0.01, *** p<0.001 when compared with Control using One way ANOVA followed by Dunnet's "t" test. Results were expressed as Mean±SEM. Here Doses *U.L.* are 250mg/kg,500 mg/kg.P<0.05,compared with control; Results were analysed by one-way anova using Dunnett's multiple comparison test.**

Table 5: Anti Inflammatory Activity by Carageenan Induced Paw Edema Method

		Mean Paw Edema (Paw volume) (cm)				
Treatment	Dose	0 hr	1hr	2hr	3hr	6 hr
Control	10ml/kg	0.36± 0.04	0.98± 0.05	1.50±0. 05	2.75±0. 08	3.50±0. 12
T1(U.L. 250 mg/kg)	250mg/kg	0.33± 0.04	0.52± 0.04	0.58±0. 04**	0.71±0. 05**	0.85±0. 04**
T2(U.L. 500 mg/kg)	500mg/kg	0.38± 0.02	0.50± 0.02	0.61±0. 01**	0.68±0. 03**	0.76±0. 04**
Diclofenac sodium(standard	10mg/kg	0.25± 0.03	0.41± 0.04	0.48±0. 03**	0.60±0. 03**	0.73±0. 02**

(Values are in Mean±S.E.M (n=6); ns -Non Significant, *p <0.05, ** p<0.01, *** p<0.001 when compared with Control using One way ANOVA followed by Dunnet's "t" test.).

Table 6: The Effect of U.L Extract On Percentage Reduction Of Edema

		Percentage reduction of edema			
Groups	Dose	1 hr	2 hr	3 hr	6 hr
U.L.	250 mg/kg	36.53%	43.10%	53.52%	61.10%
U.L.	500 mg/kg	24.00%	37.70%	44.11%	50.00%
Standard (Diclofenac sodium)	10mg/kg	39.00%	47.90%	58.30%	65.75%

By Using Carageenan Induced Paw Edema Method

4. DISCUSSION

The effect of *urena lobata* extract was studied for the anxiolytic, antidepressant and anti-inflammatory activities. In forced swim test, rats were forced to swim in a restricted space from which they cannot escape, and are induced to a characteristic behavior of immobility. This behavior reflects a state of despair which can be reduced by several agents which are therapeutically effective in human depression. The forced-swimming test, the most-widely used tool for assessing antidepressant activity preclinically, is sensitive to the effects of all of the major classes of antidepressant drugs [31]. Immobility time is reduced by clinically-relevant doses of tricyclic and atypical antidepressants, 5-HT uptake inhibitors and monoamine oxidase inhibitors in mice and rats [32 -34]. The present study revealed that *urena lobata* leaf extarct treatment, given orally, was effective in forced-swimming test. Both *urena lobata* leaf extract and imipramine significantly reduced immobility behavior which indicates depression (Table 3 Figure 3). The Maximum immobility of time was observed with *urena lobata* 250mg/kg treated group. When further increased the dose up to 500mg/kg it did not further increased the time (Table 3 Figure 3). The "tail suspension test" has been as a facile means of evaluating potential antidepressants. Effect of U.L on tail suspension method was studied and imipramine was used as a standard drug, *urena lobata* with 250mg/kg and 500mg/kg doses were studied for the antidepressant activity. The *urena lobata* showed a very good activity which is almost similar to the standard drug at 250mg/kg (Table 4 Figure 4), Clinically effective antidepressants reduce the immobility that mice display after active and unsuccessful attempts to escape when suspended by the tail. The percentage of animals showing the passive behavior is counted and compared with vehicle treated controls. The present study revealed that *urena lobata* leaf extarct treatment, given orally, was effective in tail suspension test. *urena lobata* leaf extract significantly reduced immobility behavior which indicates depression.

Administration of diazepam significantly increased the percentage of time spent and of arm entries in open arms as compare to control group. the urena lobata leaf extract at dose (500 mg/kg) resulted in a significant increase in the percentage of time and entries into open arm , compared to control groups. the urena lobata leaf extract at dose (250 mg/kg) resulted in a significant increase in the open arm time but not entry (Table 1 Figure 1). The entries per minutes stands as of the most popular in vivo animal test currently in use. the test was further validated as an animal model of anxiety on pharmacological, physiological and behavioural grounds [35] diazepam increased the percentage of open arm entries and the time spent in the open arms [36] confirming the anxiolytic effect (Table 1 Figure 1).

It can be suggested that urena lobata leaf extarct (500 mg/kg) shows clearly anxiolytic effects similar to the standard drug as a result animal spent more time in open arm and less time in closed arm. there for behavioral alteration induced by higher dose urena lobata leaf extract (Table 1 Figure 1).

Diazepam treatment had an effects on time spent in the light area, latency to dark chamber and no. of tunnel crossings *u.l.* at dose of 500 mg/kg significantly increase of time spent in light area, latency to dark chamber and no. of tunnel crossings. light/dark box is another widely used rodent anxiety model for screening anxiolytic or anxiogenic drugs(Table 2 Figure 2). It is based on the innate aversion of rodents to brightly illuminated areas and on the spontaneous exploratory behavior of rodents in response to mild stressors that is novel environment and light [34]. Drugs induced increase in behavior in the white part of a two compartment box, in which a large white compartment is illuminated and a small black compartment is darkened, is suggested as an index of anxiolytic activity [37]. In this study, the time spent in light area, latency to enter dark chamber and tunnel crossing is an indices of anxiety (Table 1 Figure 1). The *urena lobata* leaf extract (500mg/kg) had significantly increased the time spent in light area, latency to enter dark chamber and tunnel crossing, similar to standard drug, suggesting that anxiolytic activity of U.L. leaves extract as compare to control group (Table 1 Figure 1). Inflammation response of living tissue to injury involve activation of various enzyme, mediators release, cell migration, tissue breakdown and repair. Carrageenan induced paw edema is

suitable experimental animal model for evaluation anti-edematous effect of natural product. From the result *urena lobata* leaf extract at a dose of 500 mg/kg significantly inhibited carrageenan induced edema ($p<0.05$) after 60 min. *urena lobata* leaf extarct showed a dose dependent activity but was less than that produced by indomethacin(Table 5 Figure 5).

5. CONCLUSION

It is concluded that, urena lobata leaf extract reported to possess anti anxiolitic, anti depressant and anti inflammatory property. Urena lobata leaf extract may be an effective and acceptable alternative for the treatment of anxiety, depression and inflammatory conditions. Overall results of the present investigation demonstrated that urena lobata leaf extract could be the better alternative for maintaining the anxiety, depression and inflammatory conditions. These Studies lead to the conclusion that the herbal extract of urena lobata leaf extract could be used for the treatment of anxiety, depression and inflammatory, as they are found to be potent and safe. However elucidation of exact mechanism of action and beneficial effects of these formulations need further investigation. More randomized controlled trials in different animal species and strain have to be carried out before determining the status of these drugs in the therapy of anxiety, depression and inflammatory.

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