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ANXIOLYTIC, ANTIDEPRESSANT AND ANTI-INFLAMMATORY ACTIVITY OF ETHANOLIC EXTRACT OF URENA LOBATA LEAF

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ABSTRACT

Depression is one of the major mental disorders. It affects up to 25% of women and 12% of men and is a highly chronic disorder. Anxiety disorders are marked by excessive fear (and avoidance), often in response to specific objects or situations and in the absence of true danger and they are extremely common in the general population. Inflammation is a fundamental pathologic process consisting of a dynamic complex of histological apparent cytological changes, cellular infiltration and mediator release that occurs in the affected blood vessels and adjacent tissues in response to an injury or abnormal stimulation caused by a physical, chemical, or biologic agent, including the local reactions and resulting morphologic changes; the destruction or removal of the injurious material; and the responses that lead to repair and healing. Photochemical analysis of Urena lobata leaf extract has shown the presence of potent phytochemicals like alkaloids, flavonoids, glycosides, phytosteroids, fixed oils and fats, tannins and phenols. Several authors reported that flavonoids, steroids, terpenoids, phenolic acids are known to be bioactive principles. Ethanolic extracts of the extract was subjected to toxicological studies (acute toxicity studies) in mice.

KEYWORDS: Depression, Anxiety, Inflammation, Phytochemicals.

INTRODUCTION

Depression is one of the major mental disorders. It affects up to 25% of women and 12% of men and is a highly chronic disorder. Antidepressant drugsused in the treatment of major

depressive disorders are believed to act on the central monoaminergic systems mainly 5-HT and nor-adrenergic synaptic neurotransmissions. Selective serotonin reuptake inhibitors and noradrenaline reuptake inhibitors are effective in treating most depressive episodes, but about one third of these patients show only partial or no response to the treatment. Therefore, research for new antidepressants with greater effectiveness is still desirable.

Anxiety disorders are marked by excessive fear (and avoidance), often in response to specific objects or situations and in the absence of true danger and they are extremely common in the general population. According to a recent epidemiological study, the lifetime prevalence of any anxiety disorder is 28.8%. Anxiety disorders are associated with impaired workplace performance and hefty economic costs. Anxiety is also an important component of many other psychiatric or medical conditions. Effective treatments such as anxiolytic drug therapy or cognitive behavioural therapy exist but, many patients remain untreated, experience adverse effects of benzodiazepines, or do not benefit from full symptom control. It has been estimated that 43% of anxiety sufferers use some form of complementary therapy.

Etiology and clinical manifestations

Anxiety is commonly precipitated by stress but vulnerability to stress appears to be linked to genetic factors such as trait anxiety. Many patients presenting for the first time with anxiety symptoms have a long history of high anxiety levels going back to childhood. Anxiety may also be induced by central stimulant drugs (caffeine, amphetamines), withdrawal from chronic use of central nervous system depressant drugs (alcohol, hypnotics, anxiolytics) and metabolic disturbances (hyperventilation, hypoglycaemia, thyrotoxicosis). It may form part of a depressive disorder and may occur in temporal lobe lesions and in rare hormone-secreting tumours such as phaeochromocytoma or carcinoid syndrome. Apart from the psychological symptoms of apprehension and fear, somatic symptoms may be prominent in anxiety and include palpitations, chest pain, shortness of breath, dizziness, dysphagia, gastro-intestinal disturbances, loss of libido, headaches and tremor. Panic attacks are experienced as storms of increased autonomic activity combined with a fear of imminent death or loss of control.

Investigations and differential diagnosis

In patients presenting with symptoms and clinical signs of anxiety, it is important to exclude organic causes such as Thyrotoxicosis, excessive use of stimulant drugs such as caffeine and the possibility of alcoholism or with-drawal effects from benzodiazepines. However, unnecessary investigations should generally be avoided if possible.

Treatment

Treatment for anxiety disorders often requires multiple approaches. The patient may need short-term treatment with an anxiolytic, such as a benzodiazepine, to help reduce the immediate symptoms combined with psychological therapies and an antidepressant for longer term treatment and prevention of symptoms returning.

INFLAMMATION

Inflammation is a fundamental pathologic process consisting of a dynamic complex of histological apparent cytologic changes, cellular infiltration and mediator release that occurs in the affected blood vessels and adjacent tissues in response to an injury or abnormal stimulation caused by a physical, chemical, or biologic agent, including the local reactions and resulting morphologic changes; the destruction or removal of the injurious material; and the responses that lead to repair and healing.

Cardinal signs of inflammation are rubor, redness; calor, heat (or warmth); tumor, swelling; and dolor, pain; a fifth sign, functiolaesa, inhibited or lost function, is sometimes added. All these signs may be observed in certain instances, but none is necessarily always present.

Acute inflammation - starts rapidly (rapid onset) and quickly becomes severe. Signs and symptoms are only present for a few days, but in some cases may persist for a few weeks.

Chronic inflammation - this means long-term inflammation, which can last for several months and even years. It can result from:

• Failure to eliminate whatever was causing an acute inflammation

• An autoimmune response to a self antigen - the immune system attacks healthy tissue, mistaking it (them) for harmful pathogens.

• A chronic irritant of low intensity that persists.

However, chronic inflammation can eventually cause several diseases and conditions, including some cancers, rheumatoid arthritis, atherosclerosis, periodontitis and hay fever. Inflammation needs to be well regulated.

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.

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.

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.

The ethanolic extract obtained from the above extraction processes was analyzed for different phytoconstituents present in this by the method of qualitative phytochemical analysis.

ACUTE ORAL TOXICITY STUDIES-OECD

Principle of the test

It is the principle of the test that based on a step wise procedure with the use of a minimum number of animals per step, sufficient information is obtained on the acute toxicity of the test substance to enable its classification the substance is administered orally to a group of animals at one of the defined doses. The substance is tested using stepwise procedure, each step using three animals of single sex (normally females). Absence or presence of compound-related mortality of animals dosed at one step will determine the next step, i.e; (i) no further testing is needed, (ii) dosing of three additional animals, with same dose (iii) dosing of three additional animals at the next lower or the next higher dose level.

Description of the method

Selection of the animal species

The preferred rodent species is rat, although other rodent species may be used. Normally females are used. This is because the literature surveys of conventional LD_{50} tests show that, although there is little difference in sensitivity between sexes, in those cases where differences are observed females are generally slightly more sensitive. So in the present study

female rats are selected. Healthy young adult animals of commonly used strains were employed. Females were nulliparous and non pregnant. Each animal, at the commencement of the dosing, were between 8 and 12 weeks old.

Housing and feeding conditions

The Temperature in the experimental room was maintained at 22^{o}c (±3^{o}c). Although the relative humidity was maintained at least 30% and preferably not exceeded 70% other than during room cleaning it was 50-60%. Lighting was artificial, the sequence being 12 hours light, 12 hours dark. For feeding, conventional laboratory diets was used with an unlimited supply of water. Animals were grouped, such that the number of animals per cage must not interfere with clear observations of ach animal.

Preparation of animals

The animals were randomly selected, marked to permit individual identification and kept in their cages for at least 5 days prior to dosing to allow for acclimatization to the laboratory conditions.

Preparation of the doses

In general test substances should be administered in a constant volume over the range of doses to be tested by varying concentration of the dosing preparation. The maximum volume of the liquid that can be administered at once depends on the size of the test animal. In rodents volume should not normally exceed 1ml/100g body weight. However in the case of aqueous solutions 2ml/100g body weight can be considered with respect to the formulation of the dosing preparation, the use of an aqueous solution/suspension/emulsion is recommended whenever possible, followed in order of preference by solution/suspension/emulsion in oil and then possibly solution in other vehicle. Doses were prepared shortly prior administration. The dose of plant extract was 2ml/100g it is selected as per guidelines.

Procedure

Administration of the doses.

The test substance was administered in a single dose by using a stomach gavage needle. In the unusual circumstances that a single dose is not possible, the dose may be given in smaller fractions over a period not exceeding 24 hours. In the present study it was not required as the dose was administered at once. 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. After 3 hours diet was given to the animals.

Number of animals and dose levels

Three animals were used for each step. The dose level used as the starting dose selected from one of four fixed levels 5, 50, 300 and 2000mg/kg body weight. The starting dose level should be that which is most likely to produce mortality in some of the dosed animals. 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 was confident of survival of the previously dosed animals. The dose level 300 mg/kg was selected.

OBSERVATIONS

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 Thereafter, for a total of 14 days, it should be determined by the toxic reactions, time of onset and length of recovery period, and may thus be extended when considered necessary. All observations were systematically recorded with individual records being maintained for each animal.

Body weight

Individual weights of animals were determined shortly before the test substance was administered and weekly thereafter. Weight changes were calculated and recorded. At the end of the test surviving animals were weighed and humanely killed.

Data and reporting

Individual animal data were provided. Additionally, all data is summarized in tabular form, showing for each test group the number of animals used, the number of animals displaying signs of toxicity, the number of animals found dead during the test or killed for humane reasons, time of death of individual animals, a description and the time course of toxic effects and reversibility.

Animal models

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 std

drug piracetam, fourth groups 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 will be 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.

Light-Dark Transition test model

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 receives normal saline, Group 2 received std drug, Group 4 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 following parameters will be recorded during the test session of 5 min: 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.

FORCED SWIM TEST

The rats of 160-180gm will be used. 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 ± 1ºC. 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 three groups received acute dose of test drugs. The total duration of immobility will be recorded during the last 6 min of the 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.

Evaluation

Duration of immobility will be measured in controls and animals treated with various doses of a test drug or standard. Dose-responses can be evaluated.

TAIL SUSPENSION TEST

Procedure

Mice weighing 20–25g will be used. Animals were transported from the housing room to the testing area in their own cages and allowed to adapt to the new environment for 1 h before testing. 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. before 30min prior to testing. For the test the mice will be 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 will be recorded for periods of 5min. Mice will be considered immobile when they hang passively and completely motionless for at least 1 min.

Evaluation

The percentage of animals showed the passive behavior will be counted and compared with vehicle treated controls, using various doses.

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.

DRUG TREATMENT

Dose Selection: Doses will be 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 - +ve control + 1st dose of extract 250 mg/kg.

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

RESULTS AND DISCUSSION

Pharmacological investigations

Anti Inflammatory Activity by Carageenan Induced Paw Edema Method.

		Percentage reduction of oedema			
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%

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.

The Percentage Reduction of Edema of the three derivatives (D1, D2 & D3) was compared with the standard drug Diclofenac sodium. The results were tabulated below.

Effect of U.L. Extract On Forced Swim Test

		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

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**

Anti anxiety activity by Elevated Plus Maze model

		No. of entries / 5min		Time spent (Sec)/5min	
Group	**Treatment**	**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.).

Anxiolytic Activity by Dark-Light Model

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.).

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.).

DISCUSSION

FORCED SWIM TEST

In FST, 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.[82] 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. 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.

TAIL SUSPENSION

The "tail suspension test" has been as a facile means of evaluating potentialantidepressants. The immobility displayed by rodentswhen subjected to an unavoidable and inescapablestress has been hypothesized to reflect behavioral despair which in turn may reflect depressive

disorders in humans. 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.

ANXIOLYTIC ACTIVITY

Elevated plus maze test

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 EXTARCT 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 EXTARCT at dose (250 mg/kg) resulted in a significant increase in the open arm time but not entry.

The EPM 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. Diazepam increased the percentage of open arm entries and the time spent in the open arms confirming the anxiolytic effect.

It can be suggested that URENA LOBATA LEAF EXTARCT (500 mg/kg) shows clearly anxiolytic effects similar to the standard drug as result animal spent more time in open arm and less time in closed arm. There for behavioural alteration induced by higher dose URENA LOBATA LEAF EXTARCT.

Light/dark transition test

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. It is based on the innate aversion of rodents to brightly illuminated areas and on the spontaneous exploratory behaviour of rodents in response to mild stressors that is novel environment and light.[85] Drugs induced increase in behaviour 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.[86] In this study, the time spent in light area, latency to enter dark chamber and tunnel crossing is an indices of anxiety. The *URENA LOBATA* LEAF EXTARCT (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.

Anti Inflammatory Activity

Carrageenan Induced Paw edema Method

Inflammation is the response of living tissue to injury. Which involve activation of arious 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. And this involves three phases, in first phase (1 hr after Carrageenan induce) involves the release of serotonin and histamine from mast cells, in second phase (2hr) is provided by kinins and the third phase (3hr) is mediated by prostaglandins, the cycloxygenase product and lipoxygenase products. From the result *URENA LOBATA* LEAF EXTARCT 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.

CONCLUSION

It is concluded that, URENA LOBATA LEAF EXTRACTreported to possess antianxiolitic, anti depressant and anti inflammatory property; Phytochemical analysis of URENA LOBATA LEAF EXTRACT has shown the presence of potent phytochemicals like alkaloids, flavonoids, glycosides, phytosteroids, fixed oils and fats, tannins and phenols. Several authors reported that flavonoids, steroids, terpenoids, phenolic acids are known to be bioactive principles. Ethanolic extracts of the extract was subjected to toxicological studies (acute toxicity studies) in mice. No toxicity symptoms and mortality were observed even with high doses of the extracts and mixture.

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 large patient populations have to be carried out before determining the status of these drugs in the therapy of anxiety, depression and inflammatory.

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