https://africanjournalofbiomedicalresearch.com/index.php/AJBR
Afr. J. Biomed. Res. Vol. 27(September 2024); 21-25

Research Article

Assessment of Citrus Maxima Leaves Extract for Antidepressant Activity on Experimental Animals

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ABSTRACT

Due to the prevalence of depression as a mental health problem, there is a need for innovative and effective treatment regimen. The aim of this study is to evaluate the ethanolic leaves extract of pomelo plant viz ***Citrus maxima***, having antidepressant potential by employing behavioral tests on Wistar albino rats. The phytochemical investigation of ethanolic leaves extract of ***Citrus maxima*** show the presence of secondary metabolites such as flavonoid, tannin, saponin, amino acid, alkaloids, coumarins, , protein and steroid respectively. The antidepressant potential of ethanolic leaves extract of ***Citrus maxima*** was investigated by employing behavioral parameters viz Force swim test (FST), Tail suspension test (TST), Locomotor activity (LA), and effect of depression was assessed by estimated serum corticosterone (CORT) level of respective animals group. Animals were divided into five major groups, Gp I treated as normal control rats and received carboxy methyl cellulose only (10ml/kg, p.o) daily for 14 days; Gp II (stress control) rats received carboxy methyl cellulose (10ml/kg, p.o) daily for 14 days and subjected to acute restraint on 13th day respectively Gp III (standard drug treatment) rats received imipramine (15mg/kg, p.o) daily for 14 days and subjected to acute restraint on 13th day respectively; similarly the rats of Gp IV and Gp V received 200 mg/kg, p.o and 400 mg/kg, p.o of EECM daily for 14 days and subjected to restraint stress on 13th day respectively. Results revealed that the administration with leaves extract from *Citrus maxima* lowered immobility time by a substantial amount, dose-dependently, in both the FST and TST. More research is required to determine the underlying mechanisms of action, as well as to assess the safety and efficacy of the extract in treating human depression

Keywords: Acute restraint stress, Forced swim test, Tail suspension test, Locomotor activity, Imipramine

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Receiving date: 10/07/2024 Acceptance date: 20/08/2024

DOI : https://doi.org/10.53555/eewac804

INTRODUCTION

In current scenario among the biggest and most significant global public health problem is depression this serves as a serious and widespread medical disorder that has an impact on one's physical health, emotions, and thinking processes. Sadness, a lack of interest in life, guilt-related sentiments, and trouble sleeping, debilitating, fatal diseases are affected, and the number of fatalities and morbidities is rising.

The therapeutic options for depression that are now accessible are frequently accompanied by a number of unfavorable side effects and are only successful in a small percentage of individuals. Over the past ten years, there has been a tremendous advancement in the hunt for innovative

pharmacotherapy derived from medicinal plants for mental diseasess worldwide. The selective plant *Citrus maxima* is based upon the review of literature. The herb has not been fully tested to support its traditional claims of antidepressant effectiveness, according to a review of the literature. It is relevant to assess the antidepressant effects of several extracts of *Citrus maxima.*

Citrus maxima is a perennial tree which belongs to the family Rutaceae. The plant is commonly known as Pomelo, Chakotra, Bhogate, Pompela, Madhukarkati, etc. in various parts of the world. The plant is indigenous to Asia and commercially grown in many Asian countries.The plant is well distributed throughout India. It require temperature in the range of 25-32 degree celsius and rainfall in the range of 1,500-2,500 mm for its growth. It raises well in fertile soil.

The leaf of *Citrus maxima* was found to have antimicrobial, antioxidant and anticancer properties. Furthermore, the fruit juice prepared from the pulp is rich in antioxidant and polyphenolic compounds.

The aim of the present study was to evaluate the possible antidepressant effect of aqueous extract of *Citrus maxima* leaves on wistar albino rats.

MATERIALS AND METHODS

Plant material

Citrus maxima leaves were collected from Madhavpuram of Meerut, Uttar Pradesh, India. The microscopical identification of this plant was verified by Dr. Vijay Malik, Deparment of botany, Chaudhary Charan Singh University, Meerut, Uttar Pradesh, India and specimen of fruit sample was submitted as under voucher/ref. no Bot/PB/683 on dated 16/04/2024.

Extraction

Firstly the leaves were washed with double distilled water, shade dried and then powdered. 500g of the powdered material of *Citrus maxima* was extracted with 70% ethanol for 72h in a cold percolation method. After that the solvent was evaporated under pressure using Rota vapour. Final dark green residue was obtained which is used for further experiments**.**

Drug and Chemicals

All the drug solutions were freshly prepared before use. Imipramine was purchased from S.S Drugs Medical Store, Meerut, Uttar Pradesh, India. Ethanol and distilled water, carboxymethyl cellulose were obtained from Translam Institute of Pharmaceutical Education and Research Meerut, Uttar Pardesh, India. The selection of standard dose of imipramine (15mg/kg/p.o) was done on the basis of literature survey.

Experimental animals

The research protocol was initiated after taking the approval from the IAEC of Translam Institute of Pharmaceutical Education and Research, Meerut, Uttar Pradesh. All guidelines and procedures were followed according to the CCSEA, New Delhi, India respectively. The albino wistar rats weighing 180-220g, were procured from the animal house of the Translam Institute of Pharmaceutical Education and Research, Meerut, Uttar Pradesh. The animal manifested to the natural cycle of 12 hours of light 12 hours dark with optimum temperature conditions 22-24^0. The rats were kept individual cages with food pellets and water and lebitum and 7 days acclimatization periods offered to all the respective groups.

Acute restraint stress procedure

Rats were kept in separate plastic rodent restraint devices for 12 hours to perform ARS. This prevented the animal from moving physically without hurting it. Food and drink were withheld from the animals for the duration of their stress exposure. The animals were taken out of their enclosure after 12 hours, and 40 minutes later they were tested behaviorally and then given biochemical readings. Rats were housed in the experimental room's animal cage in the usual control group **(Kanase *et al.*, 2019).**

ANTIDEPRESSANT ACTIVITY

Forced Swim Test (FST)

After the application of ARS on 13th day in the evening hours. On next day (14th day) after 40 minutes rats were placed in a pool of water, and the rat's motoric activity was monitored to calculate the length of time the rats were immobile. Water was poured into a glass cylinder that had a 25 cm diameter and a height of 23 cm. Water had a 23 ± 1 ºC temperature. The appropriate dosage of the extract was administered to each rat orally. The test was administered to the animals after waiting for thirty minutes. The animal was given two minutes to become used to the new surroundings before the six minutes of measurement started. After two minutes, circumstances of increasing motor activity and periods of inactivity were alternated and assessed. For the next four minutes, immobility duration was timed using a stopwatch **(H.S.Sheik *et al.*, 2011).**

Tail Suspension Test (TST)

After the application of ARS on 13th day in the evening hours. On next day (14th day) after 40 minutes the second method for determining the antidepressant effect of tail-suspension test. The test was performed thirty min after the oral administration of ethanolic leaves extract of *Citrus maxima*. The arrangements of tail suspension test was done in between two metal tripods at a height of 70 centimeters, a cord approximately 50 centimeters long was stretched. The sticky tape was used to attach the rat's tails to the cord. After the underlying time of enthusiastic engine action, the rodents turned out to be still and the fixed status time was estimated with a stopwatch, for a complete length of 4 minutes. When rats hung motionless and passively, they were considered immobile **(H.S.Sheik *et al.*, 2011).**

Locomotor Activity

After the application of ARS on 13th day in the evening hours. On next day (14th day) after 40 minutes rats were grouped and given medication in order to study the locomotor activity with the use of an actophotometer. After 40 minutes of medication treatment, each animal was put separately in an actophotometer, and the baseline activity score of each animal was recorded for 10 minutes. **(V.K.Potdar et al; 2011))**

Collection of blood sample for serum corticosterone

Blood was drawn from the retro-orbital plexus of Wistar rats for the evaluation of corticosterone levels in blood serum. The

biochemical estimation of serum corticosterone was out sourced from Max Path Lab. The out sourced lab used ELISA method for the determination of serum corticosterone level in the blood sample.

Experimental Design

A total of 30 albino wistar rats are used for the experiment. The weight of the albino wistar rat was 180-220g and the male albino wistar rat was used for the experiment. The experimental animals were separated in 5 different groups, each group consisting of 6 animals. The division as experimental design is given below:

Group I (Normal control group)

The animals of this group was served as a normal control that neither be stressed by any method and nor treated with any drug or test compound except vehicle. Group I received a of (10ml/kg,b.w;/p.o) of 0.5% carboxy methyl cellulose dissolved in distilled water as a vehicle at a fix time once a day for 14day.**(. H.S. Sheik *et al* 2014)**

Group II Acute Restraint Stress Paradigm (Stress control)

The animals of this group was served as stress control. The depression was induced by acute restraint stress paradigm and also received (10ml/kg, b.w; /p.o) of 0.5% carboxy methyl cellulose dissolved in distilled water as a vehicle at a fix time once a day for 14days.

Group III (ARS + Imipramine treatment)

The animals of this group was served as standard control group. The acute restraint stress paradigm were utilized to induced depression along with standard drug imipramine. The selected dose of imipramine (15mg/kg, b.w/p.o) was administered after dissolving in 0.5% carboxy methyl cellulose dissolved in distilled water at a fix time once a day at a fix time interval of 14 days **(Kanase *et al.*, 2020)**.

Group IV (ARS + Low dose extract treatment)

This group of animals was treated with low dose ethanolic leaf extract of *Citrus maxima* (200mg/kg,b.w/ p.o) mixed with 0.5% carboxy methyl cellulose for the fix time of interval two times a day for 14 days and acute restraint stress paradigm were utilized to induced depression **(H.S. Sheik *et al* 2014)**

Group V (ARS + High dose extract treatment)

This group of animal was treated with high dose ethanolic leaf extract of *Citrus maxima* (400mg/kg, p.o) mixed in 0.5% carboxy methyl cellulose daily for the fix time of interval two times a day for 14 days and acute restraint stress paradigm were utilized to induced depression **(H.S. Sheik *et al* 2014)**

Statistical analysis

All values are expressed as mean±SD .Data was analyzed by One way ANOVA followed by Dunnett's t-test. ***p<0.001 and *p<0.05, $^{@}$versus normal control group and $^{\#}$versus ARS group respectively.

Results

Effect of ethanolic leaf extract (200 and 400mg/kg/p.o) *Citrus maxima* on Forced Swim Test

Effect of (200mg/kg; p.o) and (400mg/kg; p.o) of EECM two times a day on immobility time of force swim test in albino wistar rats.The results showed that the immobility time in FST of ARS group was highly significant ($^{***@}$p<0.001) compared to normal control group and Imipramine+ARS group or it was indicated that Imipramine significantly ($^{***\#}$p<0.001) reduced immobility time in FST, whereas both the doses EECM (200mg/kg; $^{*\#}$p<0.05) and EECM (400mg/kg; $^{***\#}$p<0.001) showed a dose-dependent significant reduction in immobility time when compared against ARS group respectively.

Effect of ethanolic leaf extract of *Citrus maxima* on Tail Suspension Test

The results of the test showed that the immobility time in TST of ARS group was highly significant ($^{***@}$p<0.001) compared to normal control group and Imipramine +ARS group or it was indicated that Imipramine significantly ($^{***\#}$p<0.001) reduced immobility time in TST, whereas both the doses EECM (200mg/kg; $^{**\#}$p<0.05) and EECM (400mg/kg; $^{***\#}$p<0.001) showed a dose-dependent significant reduction in immobility time when compared against ARS group respectively.

Effect of ethanolic leaf extract of *Citrus maxima* on Locomotor Activity

The results showed that the locomotor activity of ARS group was significantly lower ($^{***@}$p<0.001) compared to normal control group and Imipramine+ARS group or it was indicated that Imipramine significantly ($^{***\#}$p<0.001) improved the locomotion, whereas both the doses EECM(200mg/kg; $^{*\#}$p<0.05) and EECM (400mg/kg; $^{**\#}$p<0.001) showed a dose-dependent increment in locomotion compared to ARS group respectively

Effect of Ethanolic leaf extract of *Citrus maxima* on serum corticosterone level

One Way ANOVA followed by Benforoni multiple comparision test revealed that, Gp II had highly significant level of serum CORT (***p<0.001) compared to Gp I, III, IV and V respectively.CORT level of Gp III was found not significant (*p<0.05) compared to Gp I and Grp V, where as Gp IV CORT level was significantly higher ((*p<0.05) compared to Gp III respectively.

Table 1: Table showing the effect of Ethanolic leaf extract of *Citrus maxima* on FST, TST, Locomotor activity,and CORT respectively

S.no	Parameter/ Groups	Normal control	Stress control	Imipramine+ARS	EECM(200mg/kg) +ARS	EECM (400mg/kg) +ARS
1.	**FST**	50±5.8	107±11.5	61.1±5.10	93±9.4	73.8±10.8

2.	**TST**	65.3±13.8	115.3±6.7	73.1±4.4	92.4±7.32	80.4±8.9
3.	**Locomotor activity**	85.5±10.2	26.7±6.7	74.8±10.3	41±5.93	48.5±8.74
4.	**CORT**	90.68±8.2	147.5±3.7	87.9±4.8***	117.9±7.11	106.5±5.2

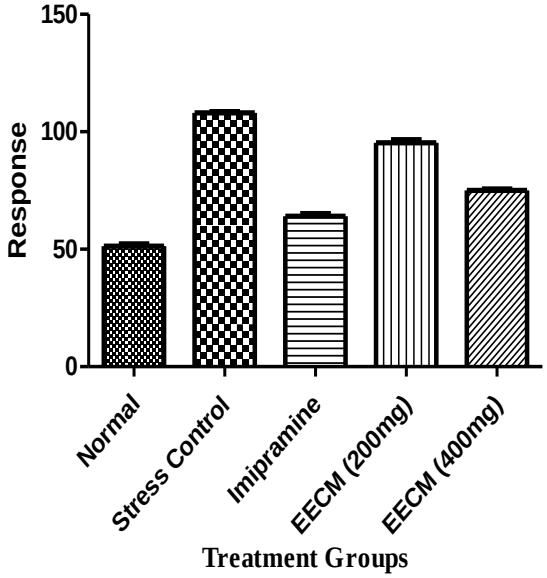

figure 1:Effect of (200mg/kg; p.o) and (1 and (400mg/kg; p.o) of EECM two times a day on immobility time of force swim test in albino wistar rats. Data represents here showed mean±SD of six animals analyzed by One way ANOVA followed by Dunnett's t-test. ***p<0.001 and *p<0.05, @versus normal control group and #versus ARS group respectively.

Discussion

In present study the 30 albino wistar rats were divided into major groupsof 5, Gp I treated as normal control rats and received 0.5% carboxy methyl cellulose solution only (10ml/kg,p.o.) daily for 14 days; Gp II (stress control) rats received 0.5% carboxy methyl cellulose (10ml/kg,p.o.) dissolved in distilled water daily for 14 day; Gp III (standard drug treatment) rats received standard drug imipramine (15mg/kg,p.o..) mixed in 0.5% carboxy methyl cellulose dissolved in distilled water daily for 14 days subjected to acute restraint on 13th day respectively; similarly the rats of Gp IV and Gp V received 200 mg/kg, p.o and 400 mg/kg, p.o mixed in 0.5% carboxy methyl cellulose dissolved in distilled water of EECM daily for 14 days and subjected to restraint stress on 13th day respectively. The acute restraint stress paradigm was used to induced depression. The main benefit of the acute restraint paradigm is that it causes unavoidable physical and mental stress without eliciting a conditioned reaction **Kanase *et al.*, 2019**. The other parameters like Force swim test (FST) and Tail suspension test (TST) are well-established tests for assessing depressive-like behavior, as they measure the response to a stressor. The result of the present study showed that oral administration of low (200mg/kg/p.o) and high (400mg/kg/p.o) dose of ethanolic leaves extract of *Citrus maxima* significantly (***p<0.001; *p<0.05) reduced immobility time in FST and TST compared to Gp II respectively (Fig.3.2 and Table 3.1). The Gp IV and V significantly (***p<0.001; **p<0.01; *p<0.05) improved the locomotion of animals compared to Gp II in a dose dependent manner respectively. Similarly in SPT test a highly significant (***p<0.001) sucrose consumption was found in Gp IV & V compared to Gp II respectively (Fig 3.4 and Table 3.1). We also measured the serum CORT level in our study Gps, low (200mg/kg/p.o) and high (400mg/kg/p.o) dose of ethanolic leaves extract of *Citrus maxima* and significantly reduced the serum CORT (***p<0.001), (*p<0.05) level in Gp IV and V as compared to Gp II respectively.. The antidepressant potential of ethanolic leaves extract of *Citrus maxima* may seems due to the presence of plant secondary metabolite viz. flavanoid and saponin repectively. Many past conducted study revealed the antidepressant potential of above mentioned phytoconstituents. So, the study highlights the potential benefits of EECM in preventing or mitigating the effects of stress on the brain's hippocampal region. The results of the behavioral parameters conducted for evaluation of antidepressant potential of ethanolic leaves extract of *Citrus maxima* may followed the conventional antidepressant mechanism by increasing the availability of mono amines neurotransmitter in the brain like serotonin, norephineprine and dopamine**.**

Conclusion

The investigation of the antidepressant potential of fruit *Citrus maxima* in Wistar albino rats provides promising evidence.

The observed behavioral changes, including reduced depressive-like behavior and increased locomotor activity, suggest that the ethanolic extract of leaves may hold potential as a natural antidepressant agent. Additional investigation is required for complete comprehension the underlying mechanisms, evaluated its efficacy and safety in humans, and establish the optimal dosage and long-term effects. Successfully, the ethanolic extract of leaves could potentially serve as a complementary or alternative treatment for individuals suffering from depression, offering a natural and potentially effective option to improve mental well-being.

Acknowledgement

Authors are thankful to Translam Institute of Pharmaceutical Education and Research, Meerut,Uttar Pradesh, India for providing the necessary support and the facilities for research.

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