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EVALUATION OF ANTIDEPRESSANT POTENTIAL OF ETHYL ACETATE PEEL EXTRACT OF GREEN AND YELLOW *CITRUS LIMON*

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ABSTRACT

Objective: *Citrus limon*, a perennial tree of family Rutaceae is known for its pharmacological importance such as anti-microbial, anti-fungal, anti - carcinogenic, anti - neurodegenerative diseases (such as alzheimer's, depression, etc.). The present study was designed to evaluate the role of the bioactive components of *Citrus limon* in treatment of neurodegenerative diseases. In this study, the antidepressant activity of ethyl acetate peel extract of yellow and green lemon was accessed in animal models. **Method:** Animal models were studied in mice. Forced swimming test (FST) and tail suspension test (TST) were used to determine the antidepressant effect of 200 and 400 mg/kg peel extracts by oral administration using imipramine (30 mg/kg) as standard drug. **Result:** The results revealed significant reduction in the immobility time of both the doses, 200 mg/kg and 400 mg/kg ethyl acetate peel extracts of yellow and green lemon. Antidepressant effect was found significant in comparison to vehicle or control ($P<0.001$), and showed similar results as that of standard imipramine drug. **Conclusion:** The results suggest that ethyl acetate peel extract of *Citrus limon* showed antidepressant like effect in mice, which can be attributed to its bioactivity. It may serve as an alternate therapeutic drug designing approach for depression, thus further investigations should be done to explore its pharmacological potential as an antidepressant.

KEYWORDS: *Citrus limon*, Peel extract, Antidepressant activity, Forced swim test (FST), Tail suspension test (TST), Immobility time, Neuropharmacological significance.

INTRODUCTION

Mental and neurological disorders have emerged as one of the leading global health concerns during the 21st century.[1] Depression is one of the most common mental disorder which is characterized by reduced interest, energy and concentration, loss of feelings, loss of appetite, poor sleep, etc.[2,3] It adversely affects interpersonal relationships, disrupts social life and career, sense of self-worth which ultimately leads to severe dysfunctioning. One of the major causes underlying depression and other bipolar disorders could be attributed to oxidative stress. Oxidative stress (OS) is the phenomenon which is associated with pathogenic mechanisms of various diseases including psychological and neurodegenerative diseases.[4] OS occurs as a result of increased concentration and harmful effects of reactive oxygen species (ROS) and reactive nitrogen species (RNS) such as hydrogen peroxide, nitric oxide, hydroxyl and superoxide radicals which causes alterations in various biological pathways and leads to their dysfunctioning.[5,6] Antidepressant drugs such as MAOIs, SNRIs, SSRIs, TCAs have been widely used to treat the conditions of oxidative stress leading to depression. Current antidepressant therapy is associated with several side effects due to which alternative approaches of new therapeutic polyherbal drug formulations are being developed. For this purpose, research on herbal and medicinal plants has constantly progressed worldwide so as to demonstrate the pharmacological effectiveness of plant species in a variety of animal models.[7] The medicinal plants containing antioxidant properties are constantly employed as an alternative source of treatment to mitigate the diseases related to oxidative stress.[8,9] *Citrus limon*, a perennial tree of family Rutaceae is one such species known for its pharmacological importance. It is an important commercial fruit crops widely cultivated in tropical and sub tropical climate. *Citrus limon* tree fruits throughout the year with fruits of deep green color on appearing and on ripening changes to bright yellow color. Lemon possess properties like antimicrobial,[10] antifungal,[11] anticarcinogenic,[12] antineurodegenerative diseases such as depression,[13] alzheimer's [14] etc. Lemon oils are beneficial for the neuroprotective activity.[15] *Citrus limon* possess cytotoxic,[16] antimicrobial [17] and anti oxidative stress [18] properties. The peel of Citrus fruits is a rich source of flavonoid glycosides, coumarins and volatile oils.[19] Apigenin present in lemon peels shows antidepressant activity.[20] Thus, literature study suggests that Citrus genus possess promising neuropharmacological potential and there exists a need to explore the pharmacognostic and biological aspects so as to isolate the neurologically active components that could be introduced in clinical practice after suitable toxicological investigations.[21] Therefore, the present study was done for the first time to investigate the antidepressant activity of the ethyl

acetate peel extracts of yellow and green *Citrus limon* with the help of FST and TST animal models.

MATERIALS AND METHODS

Plant Material: Lemon peels were collected from the local market and fruit juice shops of Bhopal, M.P., India. Peels were washed, cleaned and shade dried at an ambient temperature for 12-15 days. Dried lemon peels were first coarsely powdered using a mortar and pestle and then were further grinded using a mechanical blender.

Preparation of Ethyl Acetate Extract: The plant extract was prepared using maceration technique in which the grinded peels were kept in different solvents on the basis of increasing polarity from pet ether, chloroform, ethyl acetate to methanol. The solvents were then subsequently evaporated using soxhlet apparatus and the extract obtained was stored in refrigerator for further investigations.

Experimental Animals: To study the antidepressant activity of ethyl acetate peel extract of both yellow and green lemon, swiss albino mice (of either sex) were used, preferably non-pregnant female mice as they were found more sensitive than male mice.[22] A total number of 6 mice were used for each model studied. The animals were randomly selected as per OECD guidelines,[23] marked for individual identification and allowed to acclimatize to the laboratory conditions. All the *in vivo* experimental protocols were approved by Institutional Animal Ethics Committee (IAEC) and swiss albino mice were procured from Central Animal Facility (CAF) of PBRI institute, Bhopal.

Drugs & Chemicals Used: Saline, distilled water, 30 mg/kg body weight imipramine drug as reference standard, etc.

Experimental Design: The study of antidepressant like effect of ethyl acetate extract lemon peels was done using two animal models: Forced Swim Test (FST) and Tail Suspension Test (TST).

Screening Methodology for Antidepressant Activity: Animals were divided into four groups of six animals each for both yellow as well as green lemon peel extract.

Group I – Control (Vehicle treated group, p.o).

Group II – Standard (Imipramine 30 mg/kg, p.o in FST, TST models).[24]

Group III – Low dose of ethyl acetate peel extract of yellow lemon (200 mg/kg, p.o).

Group IV – High dose of ethyl acetate peel extract of yellow lemon (400 mg/kg, p.o).
Group III and Group IV for similarly treated doses was repeated for ethyl acetate peel extract of green lemon.

Acute Oral Toxicity: Toxicity studies were carried out on Swiss albino mice weighing between 20-30 g, as per OECD guideline No. 423.[23] Administration of doses, mode of administration, dose levels, methodology and toxicity observations were carried out as per OECD guidelines.[23] Four groups of mice comprising three animals each were treated with 5, 50, 300 and 2000 mg/kg body weight of the extract orally by gavage needle. The animals were then observed continuously for the first 4 hrs for any behavioral changes and mortality if any after 72 hrs. In acute toxicity studies, single dose of drug is given in large quantity so as to determine immediate effect for toxicity. Acute toxicity studies are done to determine LD50 values of drug or chemicals.

Forced Swim Test (FST): The FST was carried with minor modifications in the previous described experiment. A narrow glass inescapable cylinder (13 cm in diameter *24 cm high) containing water (25ºC) to a depth of 10 cm, was taken for the experiment. All the animals were fasted for 3 hours prior to the administration of vehicle/ standard/ test extracts. After thirty minutes, the mice were subjected to swim. Test was carried out for 6 minutes and the immobility time was recorded. Immobility time was the time during which the animal floated on the water surface with front paws together and remained still. The immobility time for the first two minutes was ignored as the animal was allowed to adjust to the new surrounding conditions and for the next four minutes the immobility time that alternated with conditions of enhanced motor activity was recorded with the help of a stopwatch.[25]

Tail Suspension Test (TST)

The standard drug and test extracts were orally administered, thirty minutes prior to testing. The mice were suspended on the edge of a shelf 58 cm above the table top by adhesive sellotape placed approximately 1 cm from the tip of tail. The duration of immobility was recorded with the help of stopwatch. Immobility time for the first two minutes was ignored as the animal was allowed to adjust to the new surrounding conditions and the immobility time for the next four minutes that alternated with conditions of enhanced motor activity was recorded. Mice were considered immobile when they hang passively and completely motionless.[26]

STATISTICAL ANALYSIS

Results were presented as Mean ± SD. The data was subjected to statistical analysis by One way analysis of variance (ANOVA) followed by Bonferroni's test and P<0.05*, 0.01** and 0.001*** were considered as significant, P>0.05 was considered as non-significant (NS) v/s Control group.

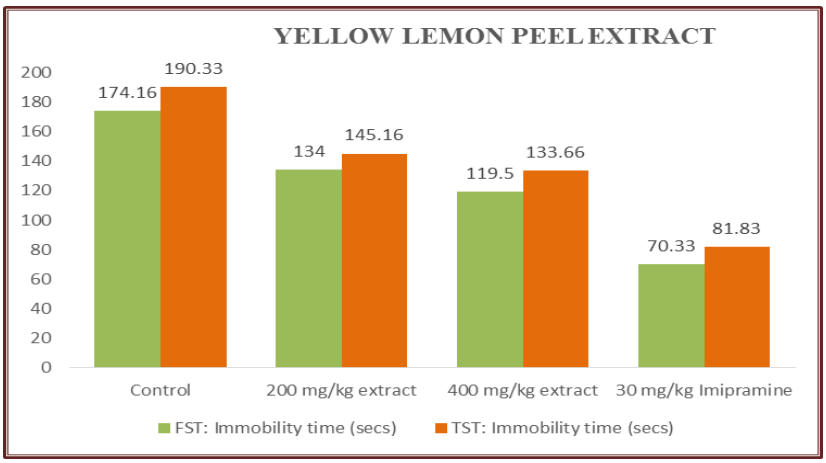
RESULTS & DISCUSSION

Table. 1: Study of immobility time in Tail Suspension Test (TST) and Forced Swim Test (FST) depression models for mice in ethyl acetate peel extract of yellow lemon.

S. No.	Treatment	TST	FST
1	Control	190.33 ± 7.11	174.16 ± 6.64
2	200 mg/kg Extract	145.16 ± 15.84***	134 ± 17.86***
3	400 mg/kg Extract	133.66 ± 12.35***	119.5 ± 16.71***
4	30 mg/kg Imipramine	81.83 ± 8.68***	70.33 ± 12.25***

*Data is Mean ± SD; *** p<0.001 compared with control by One Way ANOVA followed by Bonferroni's Test. P>0.05 was considered as non-significant (NS) v/s Control group. Number of animals for each group= 6.*

Figure. 1: Comparison of immobility time in TST and FST models of depression of yellow lemon ethyl acetate peel extract.



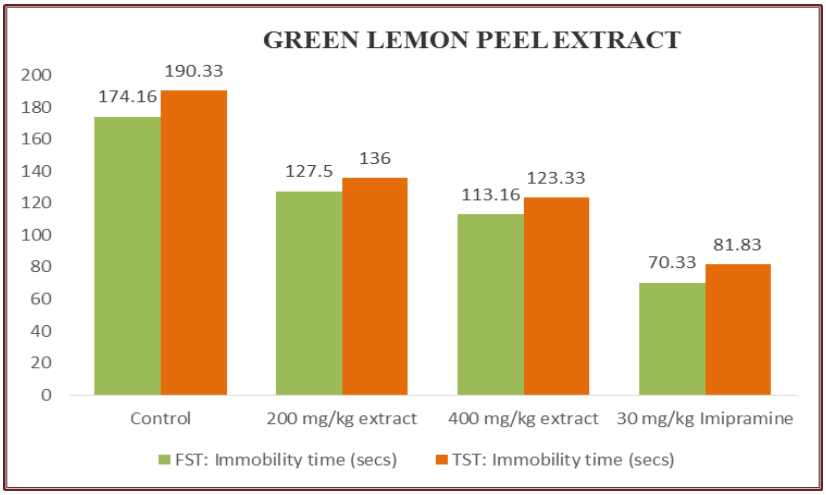
Graphical representation of immobility time in TST and FST mice models of depression in yellow lemon peel.

Table. 2: Study of immobility time in Tail Suspension Test (TST) and Forced Swim Test (FST) depression models for mice in ethyl acetate peel extract of green lemon.

S. No.	Treatment	TST	FST
1	Control	190.33 ± 7.11	174.16 ± 6.64
2	200 mg/kg Extract	136 ± 17.29***	127.5 ± 16.00***
3	400 mg/kg Extract	123.33 ± 11.84***	113.16 ± 8.63***
4	30 mg/kg Imipramine	81.83 ± 8.68***	70.33 ± 12.25***

*Data is Mean ± SD; *** p<0.001 compared with control by One Way ANOVA followed by Bonferroni's Test. P>0.05 was considered as non-significant (NS) v/s Control group. Number of animals for each group= 6.*

Figure. 2: Comparison of immobility time in TST and FST models of depression of green lemon ethyl acetate peel extract.



Graphical representation of immobility time in TST and FST mice models of depression in green lemon peel.

Acute oral toxicity studies suggests that all fixed doses of 5, 50, 300 and 2000 mg/kg body weight of the ethyl acetate peel extract of yellow as well as green lemon were found safe as no mortality was observed even at the chronic dose of 2000 mg/kg when administered orally. The results revealed that there was a significant reduction in the immobility time of both FST and TST models of depression in both doses i.e. 200 mg/kg and 400 mg/kg of ethyl acetate peel extract of yellow and green lemon in comparison to control (P<0.001). The results were found comparable with the standard drug used, 30 mg/kg imipramine. The duration of immobility behavior can be related to the despair thinking and the depression-like effect. The duration of swimming in case of FST model, is significantly enhanced with the treated doses of the ethyl acetate peel extract of both yellow as well as green lemon in comparison to the

negative control (vehicle treated group), and it also has comparable effect as that of positive control group, imipramine. Significant reduction in immobility time was observed for FST as compared to TST. The immobility time duration of both the animal models of yellow lemon is shown in Table 1, whereas immobility time of green lemon is shown in Table 2. Comparisons by the graphical representation of immobility time in TST and FST mice models of depression of yellow lemon peel extract is shown by Figure 1 and the green lemon peel extract is indicated by Figure 2.

CONCLUSION

Citrus species are widely employed as herbal medicine and aromapathy for neurological conditions like insomnia, nervousness, headache, epilepsy and depression. The present study for the first time evaluate the antidepressant activity of the ethyl acetate peel extract of *Citrus limon*. Literature studies suggests that no significant work has been done to explore the antidepressant activity of plant extracts, therefore, further research is necessay to investigate the mechanism of action involved in depression. *Citrus limon* possess antidepressant properties which may serve as an alternate therapeutic approach in drug designing for depression, therefore further investigations should be carried to explore its pharmacological potential as an antidepressant.

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