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EVALUATION OF ANTI-DEPRESSION ACTIVITY OF LINOCHIALAVEN EMULSION AGAINST STRESS INDUCED DEPRESSION USING ZEBRA FISH MODEL

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ABSTRACT

Adolescent depression is a prevalent mental health problem, with a prevalence of 4–5% in mid-to-late adolescence **[1]**. It is a significant risk factor for suicide, as well as social and educational difficulties. As a result, it's critical to recognise and treat this condition. In drug production for depressive disorder, an important approach is needed to resolve the current drug's side effects. Hence, the development of novel antidepressant models is a pressing need in biomedicine. The psychopharmacological function of linochiavalen emulsion has been demonstrated in zebra fish, an aquatic species with high sensitivity in screening antidepressants against Phobia induced depression.

In this study, 5 groups of 15 fish in each group were separated, and depression was induced by exposing the fish to the dark environment for 7 days, followed by the treatment with 1 % and 2% Linochiavalen emulsion (5ml/litre) to the fish tank for next 5 days , Citapralom (5 mg/litre) taken as standard. Behavioural parameters like- Circling movement, number of top enteries, and freezing episode are monitored.

The spectral analysis results showed that there was no chemical reaction between the oils, and also the phytomolecule Omega 3 faaty acid basic functional groups were confirmed through the FTIR analysis, then the antioxidant assay by DPPH method was found to be 500µg/ml. The emulsion-treated group's behavioural parameters were successfully reversed from the depression-induced group, and the histopathological test further confirmed the efficacy of the emulsion. Overall research concludes that the emulsion has good impact on stress induced depression.

Keywords: Linochiavalen emulsion, Anti-depression, FT-IR Spectral analysis, DPPH assay, Zebrafish

INTRODUCTION

Depression is a mental disorder that affects people of all ages. MDD (major depressive disorder) is a common and serious medical condition that affects how you feel, think, and act. Depression is the leading cause of global impairment, according to the World Health Organization. It is also the leading cause of death by suicide. The global rate of depression increased by 18 percent between 2005 and 2015 **[1]**.

Depression is a mental illness that affects Major depressive disorder (MDD) is a common and severe medical condition that has a detrimental impact on how you feel, think, and act. According to the World Health Organization, depression is the leading cause of global disability and leading cause of suicide deaths. Between 2005 and 2015, the global rate of depression rose by 18.

According to a recent WHO survey **[1]**, India has surpassed the United States and China as the world's most depressed country. With the majority of cases going unreported, India had the highest number of cases of anxiety, schizophrenia, depression, and bipolar disorder. With 6.5 percent of the population suffering from mental illness, it is clear that the gap between patients and the general public is widening **[2]**.

Chia seeds are the edible seeds of the *Salvia hispanica* plant. Omega-3 fatty acids, fats, sodium, potassium, calcium, and gluten-free protein are all present. Omega-3 fatty acids aid in the treatment of depression and mood swings **[3]**. Lavender oil is a volatile oil obtained by steam distillation of fresh flowering tops of *Lavandula officinalis Chaix.* Linalool, pienene, geraniol, and their esters, linalyl acetate are present. Thirty to forty percent of the oil is cineol. People who have difficulty in sleeping will profit from inhaling this essential oil **[4]**. Linoleic acid is known as an essential fatty acid since it

cannot be synthesised by our bodies and must be absorbed via food. It aids in the shaping of nerve tissue as well as the fatty layer that protects it **[5]**. With the literature support on the promising phytomolecule (Cineole, Omega fatty acid and Linoleic acid) in the selected specimen towards neurological disease aids to focus on its role in emulsion formulation against depressive illness in zebrafish model.

MATERIALS AND METHODS

Preparation of Emulsion

To prepare an emulsion, equal amount of linoleic acid, chia seed oil, and lavender oil are triturated well with tween 60 as an emulsifying agent and diluted with water. After that, the emulsion was standardised, high and low dose formulations were developed.

FTIR Analysis

The Fourier Transform Infrared Spectrometer is a useful tool for determining the types of functional groups or chemical bonds present in a given substance. The FTIR produces a signal spectrum at a sequence of discrete wavelengths. The chemical bonds present in a given compound can be calculated by elucidating the absorption spectrum. Chia seed oil, lavender oil and linoleic acid suspension was studied for FTIR spectrometer analysis by applying the standard Kbr pellet technique with a scan range from 400cm-1 with a resolution of 2cm-1. The above emulsion and individual oils were subjected to FT-IR in order to conform the functional groups present in the phytomolecules and check whether the emulsion results in chemical interaction **[6]**.

IN VITRO STUDIES

DPPH Assay

The suspension was tested using the 1,1-diphenyl-2-picryl hydroxyl (DPPH) method (lavender oil, chia seed, and linoleic acid). A 0.1mm DPPH solution in ethanol was prepared for this experiment, and 5ml of the solution was added to 1ml of suspensions at varying concentrations (5,10,15,20,25g/ml). The mixture was shaken and set aside for 3 hours at room temperature. The absorbance was measured at 517nm using a UV spectrometer. Escitalopram was used as a control, and the same procedure was followed, with Escitalopram's absorbance measured at 516nm using a spectrometer. The lower the absorbance of the mixture, the higher the free radical action. The percentage DPPH scavenging effec was calculated using the equation below **[7]**.

%DPPH RADICAL/SCAVENGING= Δ in the absorbance of standard – test sample/absorbance of standard*100

Animal And Housing

2-3 months old zebra fish were purchased from acquarium and subjected for experiment where it is kept in the 12hlight/12hrs dark for a period of 10 days to acclimatize inn laboratory conditions.

STRESS INDUCED DEPRESSION

Novel tank diving test

The water tank utilised for this study was 15 x 25 x 20 cm (width, length, and height), evenly separated into different horizontal portions with 9.3 cm height (top and bottom), and then further divided into eight rectangles (60.45 cm2) that were externally marked. The water column was preserved at a height of 18.6 cm (6.9 L).

The effects of the depressant drug wear off after a while. A video camera (Nikon D5100) was used to film the trials, which was set 35 cm distant from the tank and recorded the entire front half of the device. ANY-maze® software was used to conduct the behavioural studies.

The following values were recorded: delay (the time it takes for the animal to reach the top); quadrants crossed (n); erratic swimming (n, the number of times the fish swam in a zigzag pattern with rapid direction changes); period of freezing (s, the time the fish remained stationary); and swam distance (m) **[8-12]**.

RESULT AND DISCUSSION

Depression leads to feelings of sadness and/or a loss of interest in previously enjoyed hobbies. It can trigger a variety of emotional and physical problems, as well as a decrease in a person's ability to concentrate on daily tasks **[13]**. Chia seed oil, lavender oil, and linolenic acid were chosen based on the literature review. Tween 60 was used as an emulsifying agent in the preparation of 1% and 2% emulsions, and the functional groups of the compounds were standardised using FTIR, with the results indicating that there was no chemical interaction **(Fig 1-4)**.

3.1 DPPH ASSAY

In the DPPH assay, the reduction of DPPH, a stable free radical, is used. DPPH, a free radical with odd electrons, has a maximum absorption wavelength of 517nm (purple colour).

The DPPH radical scavenging potential of linochialaven emulsion and ascorbic acid is shown in the figure below. The percentage of DPPH radical scavenging action was measured at concentrations of 25, 50, 100, 250, and 500 g/ml. Free radical scanvenging property of the emulsion was observed and shown below in **Figure 5**.

Stress induced depression studies

In stress-induced animals, circular movement was minimal, while the low dose,

high dose, std drug treated group showed better effect than the stress control group. Similarly, the stress-induced group had more episodes of erratic movement and a longer bottom layer movement time, which were reversed by the emulsion and standard group. Based on the results of this study, it is hypothesized that emulsion of both the doses has effective role in depression. The circling movement is more common in drug-induced animals than in positive control animals, as seen in the table above. Similar behaviours, such as vertical swimming and swimming upside down, are more common in drug-treated animals than in healthy animals. The number of top entries, freezing time, and length are all higher in the optimistic control animal **(Table 1)**.

HISTOPATHOLOGICAL EXAMINATION

Brain parenchyma with no major pathological findings in the control group. There is evidence of gliosis, inflammation/necrosis, and neuronal aggregates/degeneration in the disease control community. Reactive gliosis in the low-dose community. The presence of surrounding neurona aggregates and focal neuronal degeneration/degenerated corpora has been noted. There is focal gliosis in the high-dose population **(Fig 6 a-e)**.

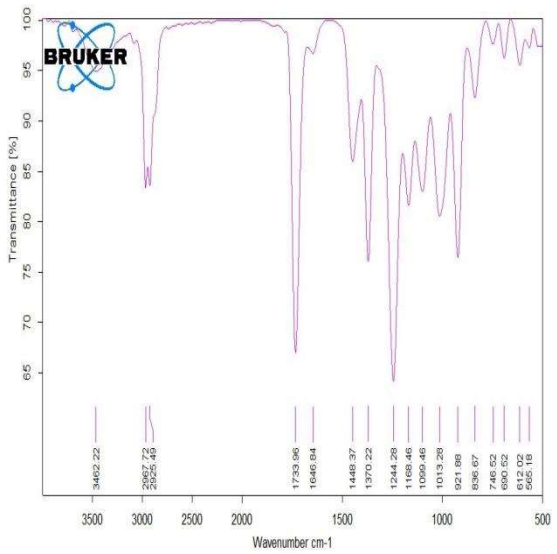


Fig 1: FT-IR analysis graph of lavander oil

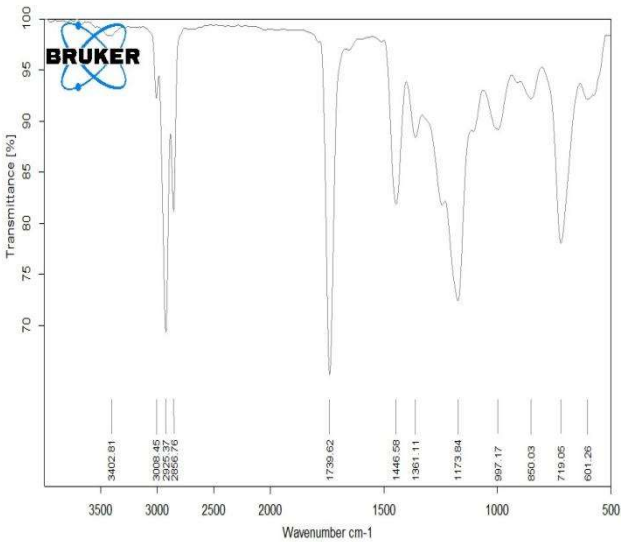


Fig 2: FT-IR analysis graph of linoleic acid

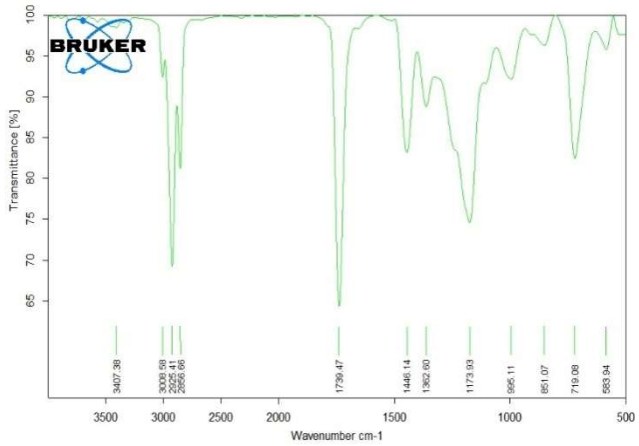


Fig 3: FT-IR analysis graph of chia seed oil

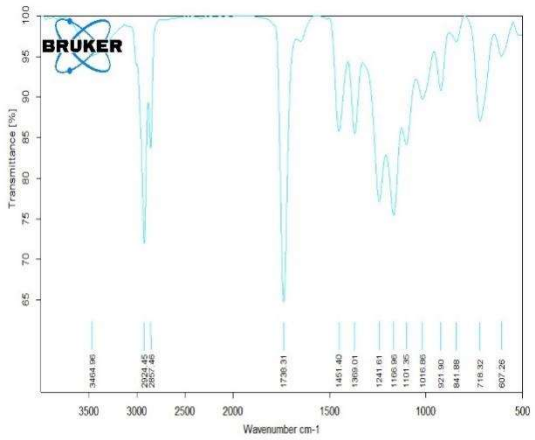


Fig 4: FT-IR analysis graph of Emulsion

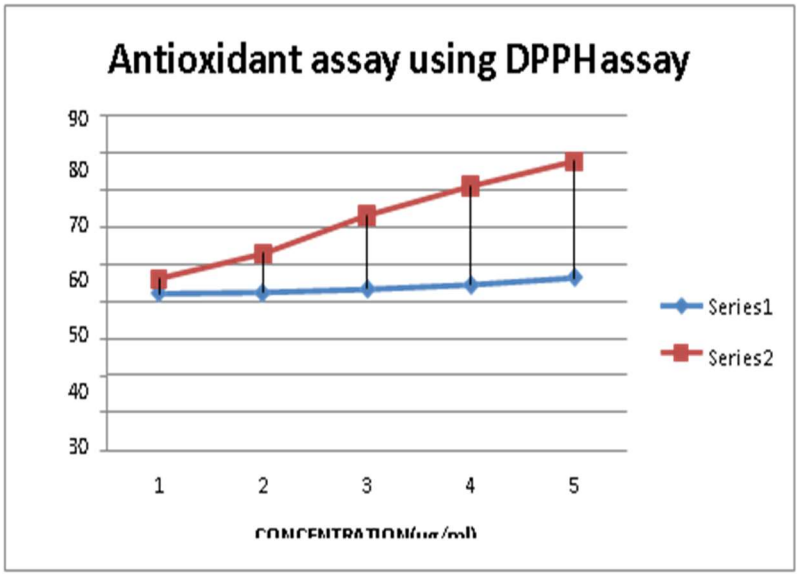
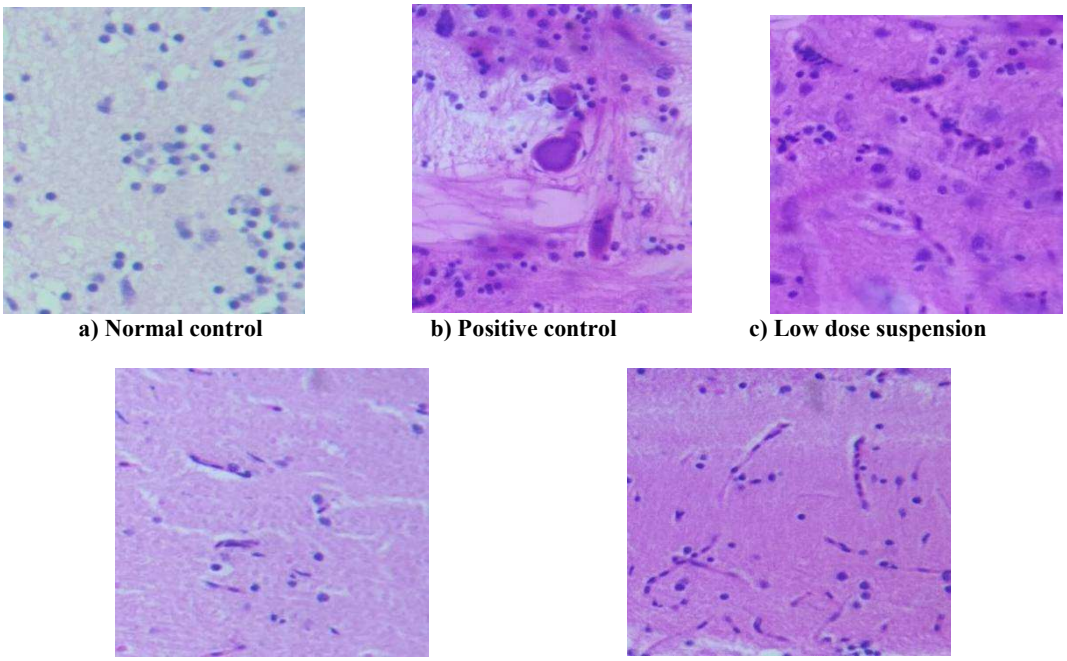


Fig 5: Study of DPPH assay on Linochialaven emulsion

Table 1: Effect of Linochialaven emulsion on stress induced behavioral parameter

Behavioural parameters	Control	Disease control	Low dose (0.1mg/ml)	High dose (0.2mg/ml)	Standard (Escitalopram 0.5mg/100ml)
Circling movement	4.25±1.02	1.75±0.66	2.2±0.01**	4.5±0.90*	4.15±0.01*
Number of top entries	4.2± 1.88	1.68±0.12	3.4±1.02*	5.75±1.24*	6.4± 2.22*
Freezing episode	1.25 ±0.54	3.6±1.02	2.2±0.98*	1.25±0.02*	1.04±0.96*
Freezing duration(sec)	12.25± 4.22	37.32± 2.40	18.5± 2.44**	13.06±4.12*	9.7± 2.08*
Erratic movement	1.54±0.08	6.25±1.22	3.24±0.96**	2.74±0.08**	1.68± 0.44*

All the datas were mentioned as Mean ± SEM calculated by One way ANOVA followed by Dunnettest.p*<0.5



a) Normal control **b) Positive control** **c) Low dose suspension**

d) High dose suspension **e) Standard treated**

Fig 6: a-e: Histopathological Examination

Linochialaven emulsion is beneficial in the treatment of depression and mood swings due to the presence of omega-3 fatty acid (polyunsaturated fatty acid with more than one double bond) in chia seed oil, which aids in the treatment of depression and mood swings by altering cell signalling and cell membrane structure, as well as increasing the amount of DHA in the brain, which is related to higher neurogenesis and better good recall and learning assessments The presence of linalool and linalyl acetate in lavander oil in suspension also helps to enhance the sleep cycle, which is beneficial in the treatment of depression and for people who have sleep disturbances. Since omega-3 fatty acid is difficult to target, linoleic acid contains omega-6 fatty acid, which is now used as a supplement. Acute Escitalopram markedly affected the adult zebra fish treated with 5mg of the standard escitalopram sample, the standard antidepressant drug Escitalopram is used to test the behaviour of animal and also to compare the activity of our prepared **[14-16]**.

Since chia seed oil contains omega-3 fatty acids (polyunsaturated fatty acids with more than one double bond), it can help with depression and mood swings by altering cell signalling and cell membrane structure, as well as the the amount of DHA in the brain, which has been linked to increased neurogenesis and better learning and memory tests **[17, 18]**.

Similarly, the presence of linalool and linalyl acetate in lavander oil in suspension helps to

boost the sleep cycle, which assists in the treatment of depression and also for people who suffer from sleep disturbances. Linoleic acid contains omega-6 fatty acid, which is often used as a substitute for omega-3 fatty acid since obtaining an adequate amount of omega-3 fatty acid is difficult.

Linoleic acid contains omega-6 fatty acid which is used nowadays as a subsitute of omega-3 fatty acid it is used in the formation of nerve tissue, and its fatty layer insulates the nerve tissue to achieve a sufficient amount of omega-3 fatty acid **[19-21]**.

CONCLUSION:

In this study, Linochavalen emulsion screened for stress induced antidepressant studies in zebrafish model has shown its better efficacy through *invitro* DPPH assay and also by s*tudies* through behavioural parameters and histopathological examination.

In the emulsion, presence of omega- 3 fatty acid in the chiaseed oil, Linalool and linalyl acetate in Lavender oil and the effect of omega- 3 fatty acid in the Linoleic acid altogether might be responsible for the antidepressant activity. Further studies are essential to prove the efficacy of emulsion clinically.

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