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Synergistic Antidepressant Effect of Clitoria Ternatea and Phyllanthus Emblica in Experimental Animal

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Abstract:

Introduction: A study on Clitoria Ternatea and Phyllanthus Emblica, is needed to investigate their potential and synergistic effect to enhance antidepressant actions. Both plants have been traditionally used for their memory-enhancing, anxiolytic, and psychiatric disorder management properties.

Aim: To evaluate synergistic antidepressant activity of Clitoria Ternatea and Phyllanthus Emblica in experimental animals.

Materials and Methods: The depression was induced in mice by using Shock-induced depression (SID) model for 8 days. Forced swimming test (FST), tail suspension test (TST) was for immobility testing were used to examine the antidepressant activity in Electric Shock induced depression in mice. The biochemical parameters like serum Corticosterone level and oxidative stress parameter like Reduced Glutathione test (GSH) and Lipid peroxidation test (LPO) were done on Day14 of treatment.

Result: The combination of both C. Ternatea 50 mg/kg with P. Emblica 2 mg/kg were shows the greater significant results in models. Combination of both plant extracts were greater significant decrease in corticosterone level in blood serum and LPO level in brain and also greater significantly increase in GSH level in Brain.

Conclusion: The co-administration of aqueous extracts of C. Ternatea with P. Emblica in a animal model showed a synergistic antidepressant impact, indicating their potential to alleviate depressive-like behaviors when used together.

Keyword: Antidepressant, Synergism, Medicinal plant, Clitoria Ternatea, Phyllanthus Emblica.

Introduction:

Depression is a common and devastating mental illness is characterized by a wide variety of emotional and physical issues, as well as enduring feelings of melancholy and disinterest in activities [1]. Millions of individuals worldwide are impacted, greatly reducing their functionality and quality of life [2].

Throughout history, traditional medical systems had utilized medicinal plants to treat a broad range of illnesses, including mental health issues. Phyllanthus Emblica, sometimes referred to as Indian gooseberry, amla, and Clitoria Ternatea, also known as butterfly pea, have drawn interest due to their possible neuropharmacological qualities [3]. Because of Clitoria Ternatea consist high concentration of flavonoids, anthocyanins, and various other bioactive substances, Clitoria Ternatea is well-known as having nootropic, anxiolytic, as well as antidepressant-like properties [4]. Phyllanthus Emblica, is recognized for its significant antioxidant and adaptogenic capabilities, chiefly due to its high vitamin C concentration and polyphenolic components [5].

In the context of herbal medicine, the idea of synergism where the combined impact of two drugs surpasses the total of their separate effects is especially pertinent [6]. Potentially synergistic interactions between Phyllanthus Emblica and Clitoria Ternatea may maximize the antidepressant benefits of both plants while reducing adverse effects [7]. Even though there many different antidepressant drugs available, these therapies frequently have drawbacks, such as delayed start of action, adverse effects, and an insufficient response in large number of individuals. Thus, the demand for safer and more effective therapy choices is urgent [2].

This study was to explore the synergistic antidepressant activity of Phyllanthus Emblica with Clitoria Ternatea using experimental animal models. For Behavioral assays in Elctrical shock induced depression in mice, Forced Swim Test (FST) as well as Tail Suspension Test (TST), are utilized in order to provide a thorough assessment of the combined effects of these medicinal plants.

2. Materials and Methods:

2.1. Aqueous extraction of Clitoria Ternatea:

The Clitoria Ternatea leaves were cleaned, shade-dried, and powdered. One hundred grams of powdered leaf sample were weighed and boiled with One liter of distilled water for 10 min. at 70ºC. The plant extract was filtered and evaporated to dryness. The extract was weighed daily, reconstituted with distilled water according to the needed dose (50 mg/kg), and administered orally by a feeding tube [4].

2.2. Aqueous extraction of Phyllanthus Emblica:

80-gram dried fruit of Phyllanthus Emblica were crushed into fine powder and extracted with One liter of boiling water for 30 min. The heated decoction was allowed to cool to room temperature before filtering twice through fine filter paper. The filtrate was then evaporated in a water bath until completely dry. The extract was brown, and the yield of the extract was 25.6%

w/w. The extract was stored in a desiccator and used for the pharmacological studies by dissolving each time in distilled water [8].

2.3. Animal

The experiment protocol was approved by K.B.H.S.S Trust institute of pharmacy, Malegaon, Dist. Nashik, Maharashtra, India. (Reg no. 1566/PO/Re/S/11/CPSEA). Thirty-six (36) albino mice (body weight: 25–35 g) of both sexes were chosen at random and placed into 6 groups (n = 6) Group I was NC or normal control group, Group II was DC or Disease control, Group III was FLU, Group IV was C. T, Group V was P. E and last group was Group VI C. T + P. E. Once they had adjusted to their new surroundings, they were kept in an animal house with a 12-hour light-dark cycle, 27±2ºC temperature, and 45–55% relative humidity.

2.4. Electric shock induced depression:

The mice were used from the KBHSS Trust's Institute of Pharmacy Animal House. Each group of mice except Normal control group or Group I NC depression was induced. Metal grid of actophotometer was used. Mice were Shocked for 1 hour. 0.75 mA of current for 5sec duration at interval of 10sec. Daily 8 days this procedure followed. Shock reduced the activity of mice it was measured in actophotometer. [9] After 8 days, all group of mice were put into the actophotometer the activity was recorded in actophotometer for a period of 10min. Note the basal activity score of all the animals. Locomotor activity was taken as any movement mice intersect the light ray's, readings increases and it was shown on digital analogue screen [10]. The results were statistically analyzed.

2.5. Experimental design

Thirty-six (36) albino mice (body weight: 25–35 g) of both sexes were chosen at random and placed into 6 groups (n = 6) each group treated for 14 days Group I was NC or normal control group with normal feed and water, Group II was DC or Disease control group with normal fed and water, Group III was Standard drug or (FLU) Fluoxetine 20mg/kg after dissolving in water oral administered with normal feed and water , Group IV was C.T or Clitoria Ternatea 50mg/kg after dissolving extract in water oral administered with normal feed and water, Group V was P.E or Phyllanthus Emblica 2mg/kg after dissolving extract in water oral administered with normal feed and water and last group was Group VI Clitoria Ternatea 50mg/kg with Phyllanthus Emblica 2mg/kg after dissolving extracts in water oral administered with normal feed and water.

2.6. Forced swimming test:

The mice were kept separately in a glass beaker of 11 cm in diameter and 15 cm in height, filled with fresh water up to 6 cm in height, and kept at a temperature of 27±2 C for 15 minutes. Day 1, Day 8, and Day 14 participants were required to swim for 6 minutes in a comparable setting during a "test-session" [11]. The test sessions were held on Days 1, 8, and 14 before to and following the medication treatment. When a mouse floats motionless or makes just the motions

required to maintain its head above the water's surface, it is said to be immobile [12]. The total duration of the immobility during the last 4 min of the 6 min test was recorded.

2.7. Tail suspension test:

Mice were suspended by tail from a height of 75cm [13]. The mouse made attempts to regain upright posture, but continued in a motion less state (immobility phase). Baseline immobility was measured for a period of 6 min. After that, the test sessions were held on Days 1, 8, and 14 following the medication treatment [14,15].

2.8. Corticosterone assay:

In order to avoid fluctuations on hormone levels due to circadian rhythm, mice were bled at 12:00 p.m. to 13:00 p.m. on the day of sacrifice. Blood was collected through Retroorbital technique and sampled into plain tubes and was kept in room temperature [16]. It was centrifuged at 3000 rpm for 10 minutes to separate the serum and red blood cells. The serum was kept frozen at −20 ◦C freezer and stored it until assayed was performed [17]. Serum corticosterone concentrations were determined using a commercially available enzyme immunoassay kits (ichroma™ Cortisol) used for the quantitative determinants of corticosterone in serum or plasma following the manufacturer's instructions.

2.9. Nonenzymic antioxidants:

2.9.1. Reduced Glutathione (GSH):

Total reduced glutathione (GSH) was determined by the method of [18]. Following TCA precipitation, the brain homogenate sample was allowed to react with DTNB and phosphate buffer. At 410 nm, the absorbance was measure [19].

2.9.2. Lipid peroxidation (LPO):

The levels of tissue thiobarbituaric acid reactive substances (TBARS) were measured using the method of [20]. The pink chromogen was measured at 534 nm [21].

Statistical analysis:

For every group, the results of these experiments were presented as mean ± standard error of mean (SEM). For all statistical studies, GraphPad PRISM version 10.0 was utilized. One-way analysis of variance (ANOVA) was used to examine intergroup differences. Bonferroni multiple comparison test was used as a post hoc test to compare the group means.

3. Results:

3.1. Biochemical parameter

Preliminary Phytochemical screening:

Preliminary Phytochemical screening of the Clitoria Ternatea and Phyllanthus Emblica revealed the presence of different chemical constituents such as alkaloids, carbohydrates, saponins, Tannins, flavonoids and steroids [4,8] in Table no.3.1 and 3.2.

Table no 3.1. Phytochemical tests for Clitoria Ternatea.

Sr.no.	Phytochemical tests for Clitoria Ternatea		Result
1	Alkaloids	Hager's test Dragendroff's test	+
2	Carbohydrates	Molisch's test	+
3	Saponins	Froth test	+
4	Tannins	Ferric chloride test	+
5	Flavonoids	Alkaline reagent test Lead acetate test	+
6	Steroids	Sulphur powder test	+

Sr.no.	Phytochemical tests for Phyllanthus Emblica		Result
1	Alkaloids	Hager's test Dragendroff's test	+
2	Carbohydrates	Molisch's test	+
3	Saponins	Froth test	+
4	Tannins	Ferric chloride test	+
5	Flavonoids	Alkaline reagent test Lead acetate test	+
6	Steroids	Sulphur powder test	+

Table no. 3.2. Phytochemical tests for Phyllanthus Emblica

3.2. Actophotometer:

The whole group of animals except normal control or group I NC, shows highly significant decrease in locomotor activity after electric shock induce depression. Group II DC ($^{***}p<0.001$), Group III FLU ($^{***}p<0.001$), Group IV C. T($^{***}p<0.001$), Group V P. E ($^{***}p<0.001$), Group VI C. T+ P. E ($^{***}p<0.001$) animal shows a significant decrease in locomotor activity in

actophotometer after induction of depression, when compared to normal control group (I NC). The '***' indicates that this reduction is highly significant.

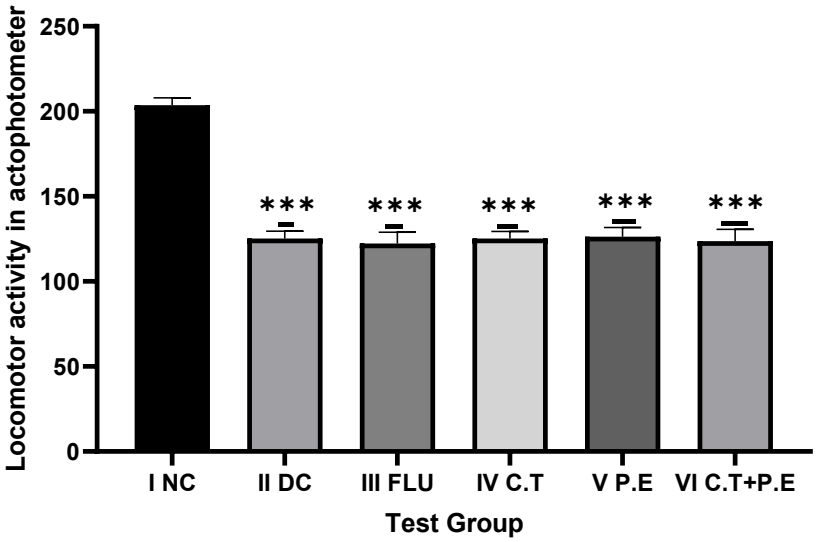

Figure no 1: Locomotor activity after induction of depression

NC - Normal control, DC - Disease control, FLU – Fluoxetine, C. T – Clitoria Ternatea, P.E – Phyllanthus Emblica and C. T+P.E - Clitoria Ternatea with Phyllanthus Emblica.
V P.E – Phyllanthus Emblica and VI C. T+P.E - Clitoria Ternatea with Phyllanthus Emblica.
Data are presented as means ± SD (n=6/group). One-Way ANNOVA followed by Bonferroni test for multiple comparison. $^{***}p<0.0001$when compared to normal control (NC).

3.3. Effect of drugs on forced swimming test:

Depressed group or Disease control group (II DC) shows significant increase in duration of immobility on Day1, Day 8 and Day 14 and Fluoxetine (20mg/kg) show significant reduction in duration of immobility ($^{***}p < 0.001$), ($^{****}p < 0.0001$) and ($^{****}p < 0.0001$) on Day1, Day 8 and Day 14. The combination of both Clitoria Ternatea 50mg/kg with Phyllanthus Emblica 2mg/kg compared with depressed group or Group II DC showing antidepressent activity because immobility time was significantly decreases in Day 8 ($^{****}p < 0.0001$), Day 14 ($^{****}p < 0.0001$). The combination of Clitoria Ternatea 50mg/kg + Phyllanthus Emblica 2mg/kg was compared to Standard or fluoxetine shows significant decrease result on Day 14 ($^{aa}p<0.01$). The combination of Clitoria Ternatea 50mg/kg + Phyllanthus Emblica 2mg/kg was compare to alone Clitoria Ternatea 50mg/kg or VI group shows significant decrease in result on Day 14 ($^{bb}<0.01$). The combination of Clitoria Ternatea 50mg/kg + Phyllanthus Emblica 2mg/kg was compared to alone Phyllanthus Emblica 2mg/kg shows significant decrees in results on Day 8 and Day 14 ($^{cc}<0.01$) and ($^{ccc}<0.001$). This combination helps to decrease the duration of immobility and also shows synergism effect of both plants as antidepressants. As compare to alone drug study, the combination of these two drugs shows higher results.
[Figure no.2].

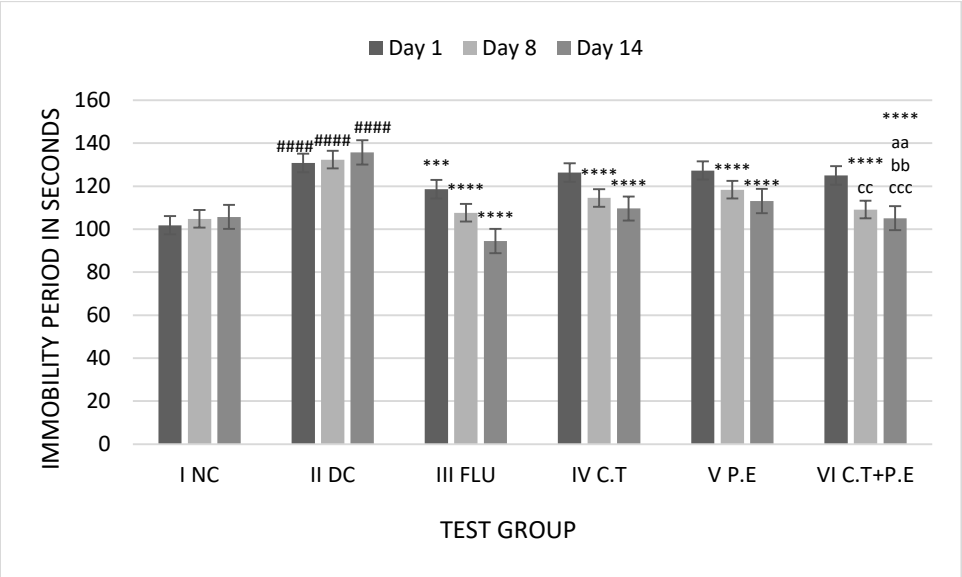

Figure no 2: Forced swim test Day 1, 8 and 14

I NC - Normal control, II DC - Disease control, III FLU – Fluoxetine 20mg/kg, IV C. T – Clitoria Ternatea 50mg/kg, V P.E – Phyllanthus Emblica 2mg/kg and VI C. T+P.E - Clitoria Ternatea 50mg/kg + Phyllanthus Emblica 2mg/kg.

Data are presented as means ± SD (n=6/group). One-Way ANNOVA followed by Bonferroni test for multiple comparison;

$^{####}p<0.0001$when compared to normal control (NC); $^{****}p<0.0001$ when compared to disease control (DC); $^{aa}p<0.01$ when compared to fluoxetine (III-FLU); $^{bb}p<0.01$ when compared to Clitoria Ternatea (IV-C. T); $^{ccc}p<0.001$ when compared to Phyllanthus Emblica (V-P. E).

3.4. Effect of drugs on tail suspension test:

The period of immobility significantly increase in the depressed or disease control group on day 1, day 8 and day14 ($^{####}p > 0.000$), ($^{####}p > 0.000$) and ($^{####}p > 0.000$) as compared with normal control group. The combination of Clitoria Ternatea 50mg/kg + Phyllanthus Emblica 2mg/kg comparison with disease group or depressed group results in an even greater decreased in immobility activity in tail suspension test on day 1 ($^{****}p<0.0001$), day 8 ($^{****}p<0.0001$) and day14 ($^{****}p<0.0001$). The combination of Clitoria Ternatea 50mg/kg + Phyllanthus Emblica 2mg/kg was compared to Standard or fluoxetine shows significant increase results on Day 14($^{aa}p<0.01$). The combination of Clitoria Ternatea 50mg/kg + Phyllanthus Emblica 2mg/kg was compare to alone Clitoria Ternatea 50mg/kg or VI group shows significant decrease in results on Day 8, Day 14 ($^{bb}p<0.01$) and ($^{bbb}p<0.001$). The combination of Clitoria Ternatea 50mg/kg + Phyllanthus Emblica 2mg/kg was compared to alone Phyllanthus Emblica 2mg/kg shows significant decrees in results on Day 8, Day 14 ($^{ccc}p<0.001$) and ($^{ccc}p<0.001$). This combination helps to decrease the immobility duration of mice and also shows synergism effect of both herbal plant as antidepressants activity. As compare to alone drug study, the combination of these two drugs shows higher results. [Figure no.3].

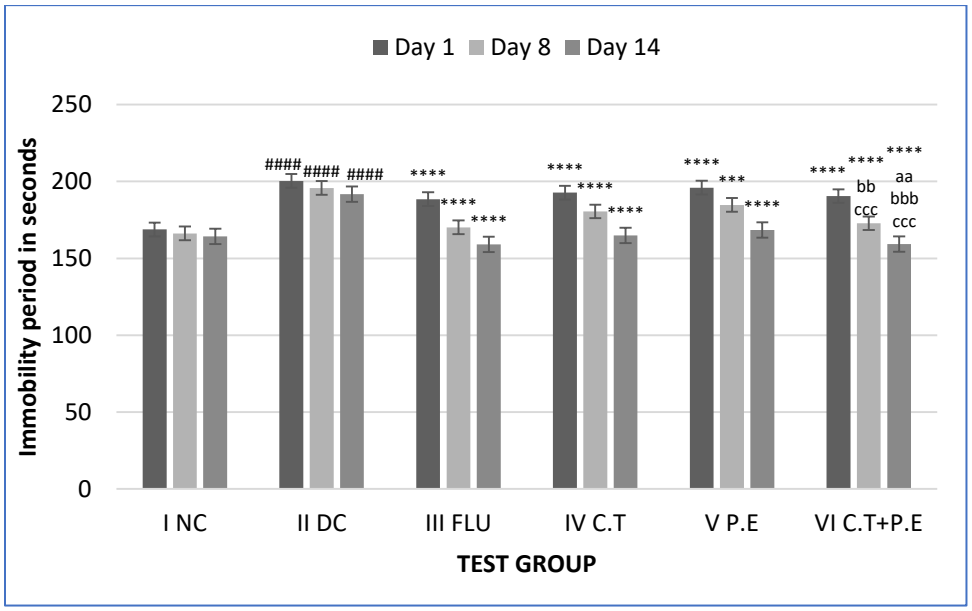

Figure no.3: Tail suspension test Day 1, 8 and 14

I NC - Normal control, II DC - Disease control, III FLU – Fluoxetine 20mg/kg, IV C. T – Clitoria Ternatea 50mg/kg, V P.E – Phyllanthus Emblica 2mg/kg and VI C. T+P.E - Clitoria Ternatea 50mg/kg + Phyllanthus Emblica 2mg/kg.

Data are presented as means ± SD (n=6/group). One-Way ANNOVA followed by Bonferroni test for multiple comparison;

$^{####}p<0.0001$when compared to normal control (NC); $^{****}p<0.0001$ when compared to disease control (DC); $^{aa}p<0.01$ when compared to fluoxetine (III-FLU); $^{bbb}p<0.001$ when compared to Clitoria Ternatea (IV-C. T); $^{ccc}p<0.001$ when compared to Phyllanthus Emblica (V-P. E).

3.5. Corticosteron assay:

The cortisol levels decrease significantly in Fluxetine 20 mg/kg group as compared with depressed or II DC goup $^{***}P<0.001$. The combination of Clitoria Ternatea 50mg/kg with Phyllanthus Emblica 2mg/kg results in an even greater decreased in corticosterone level highly significant $^{***}p<0.001$. The combination of Clitoria Ternatea 50mg/kg + Phyllanthus Emblica 2mg/kg was compared to Standard or fluoxetine shows significant increase results ($^{aa}p<0.01$). The combination of Clitoria Ternatea 50mg/kg + Phyllanthus Emblica 2mg/kg was compare to alone Clitoria Ternatea 50mg/kg or VI group shows decrease in results ($^{b}<0.05$). The combination of Clitoria Ternatea 50mg/kg + Phyllanthus Emblica 2mg/kg was compared to alone Phyllanthus Emblica 2mg/kg shows highly significant increase in results ($^{ccc}p<0.001$). This combination helps to decrease the corticosterone level in brain and also shows synergism effect of both herbal drug as antidepressants. As compare to alone drug study, the combination of these two drugs shows lower results of cortisol levels [Figure no.4].

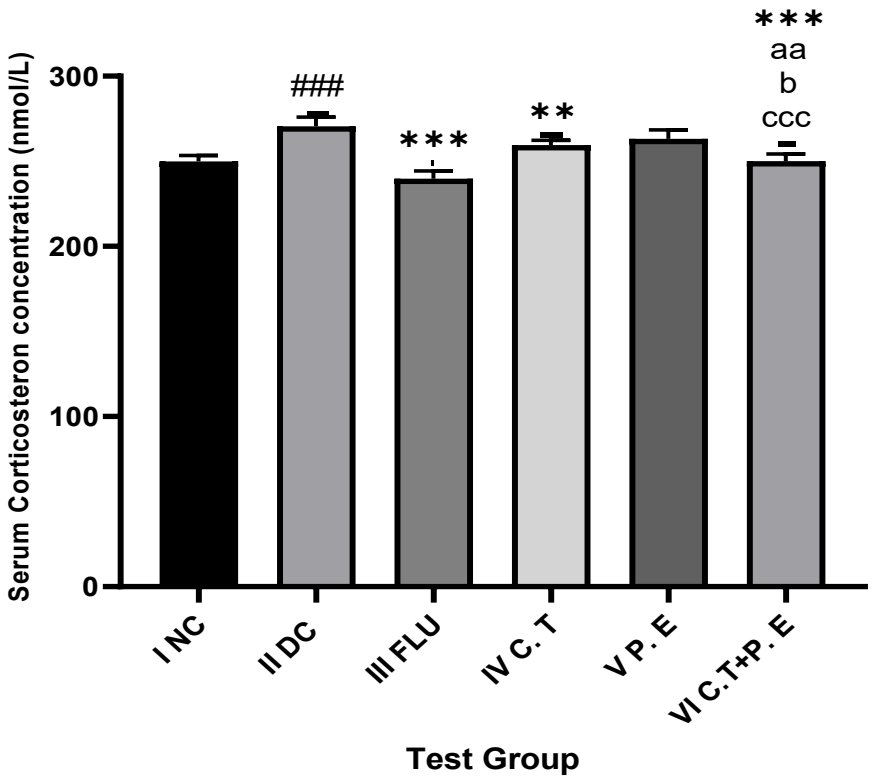

Figure no:4: Corticosterone level in blood serum of mice

NC - Normal control, DC - Disease control, FLU – Fluoxetine 20mg/kg, C. T – Clitoria Ternatea 50mg/kg, P.E – Phyllanthus Emblica 2mg/kg, and C. T+P.E - Clitoria Ternatea 50mg/kg with Phyllanthus Emblica 2mg/kg.

Data are presented as means ± SD (n=6/group). One-Way ANNOVA followed by Bonferroni test for multiple comparison;

$^{###}p<0.001$when compared to normal control (NC); $^{***}p<0.001$ when compared to disease control (DC); $^{aa}p<0.01$ when compared to fluoxetine (III-FLU); $^{b}p<0.05$ when compared to Clitoria Ternatea (IV-C. T); $^{ccc}p<0.001$ when compared to Phyllanthus Emblica (V-P. E).

3.6. Effect of Reduced Glutathione (GSH):

The amount of Vitamin C in brain of mice under different conditions was shown in Figure 5. The concentration of Vitamin C was significantly decreased in brain of group II (depressed) mice, when compared to that of group I mice. The condition was reversed during treatment. In groups IV and V, the concentration of Vitamin C was significantly increased when compared to that of group II (depressed) mice. The combination of Clitoria Ternatea 50mg/kg + Phyllanthus Emblica 2mg/kg results in an even greater increased in glutathione (GSH) level in Brain of mice highly significant ($^{***}p<0.001$) as compared to depressed or disease control group. The combination of Clitoria Ternatea 50mg/kg + Phyllanthus Emblica 2mg/kg was compare to alone Clitoria Ternatea 50mg/kg or IV group shows significant increase in result ($^{bb}p<0.001$). The combination of Clitoria Ternatea 50mg/kg + Phyllanthus Emblica 2mg/kg was compared to alone Phyllanthus Emblica 2mg/kg shows significant increase in results ($^{cc}p<0.01$). This combination helps to

increase reduced glutathione (GSH) level in brain and also shows antioxidant activity. As compare to alone drug study, the combination of these two drugs shows higher results of GSH levels. [Figure no.5].

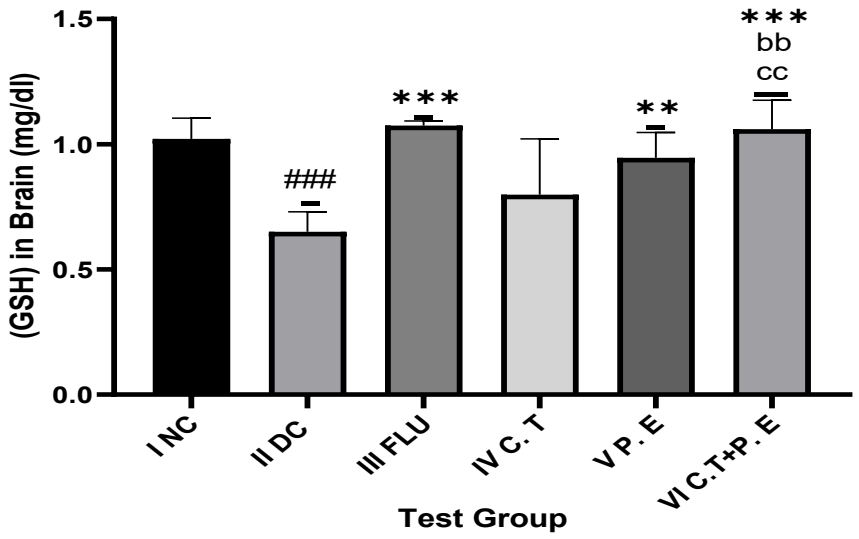

Figure no 5: Non-enzymic antioxidant Reduced Glutathione (GSH) level in brain of mice

NC - Normal control, DC - Disease control, FLU – Fluoxetine 20mg/kg, C. T – Clitoria Ternatea 50mg/kg, P.E – Phyllanthus Emblica 2mg/kg, and C. T+P.E - Clitoria Ternatea 50mg/kg with Phyllanthus Emblica 2mg/kg.

Data are presented as means ± SD (n=6/group). One-Way ANNOVA followed by Bonferroni test for multiple comparison;

$^{###}p<0.001$when compared to normal control (NC); $^{***}p<0.001$ when compared to disease control (DC); $^{bb}p<0.01$ when compared to Clitoria Ternatea (IV-C. T); $^{cc}p<0.01$ when compared to Phyllanthus Emblica (V- P.E).

3.7. Effect of Lipid Peroxidation (LPO):

Figure 6 shows the estimation lipid peroxidation in brain of mice under different conditions. In group II (depressed) mice the level of Lipid peroxidation was significantly increased when compared to that of group III. The reverse in levels was observed in the treatment groups. The level of lipid peroxidation was significantly decreased in group IV and V as that of group VI mice. The combination of Clitoria Ternatea 50mg/kg + Phyllanthus Emblica 2mg/kg results in an even greater decreased lipid peroxidation in brain of mice highly significant ($^{***}p < 0.001$) as compared with disease control or depressed group. The combination of Clitoria Ternatea 50mg/kg + Phyllanthus Emblica 2mg/kg was compared to Standard or fluoxetine 20mg/kg shows significant increase results ($^{a}p<0.05$). The combination of Clitoria Ternatea 50mg/kg + Phyllanthus Emblica 2mg/kg was compare to alone Clitoria Ternatea 50mg/kg or IV group shows significant decrease in result ($^{b}p<0.05$). The combination of Clitoria Ternatea 50mg/kg + Phyllanthus Emblica 2mg/kg was compared to alone Phyllanthus Emblica 2mg/kg shows significant decrease in results ($^{cc}p<0.01$). This combination helps to increase lipid peroxidation in brain of mice and also shows antioxidant activity. As compare to alone drug study, the

combination of these two drugs shows lower results of lipid peroxidation (LPO) level.

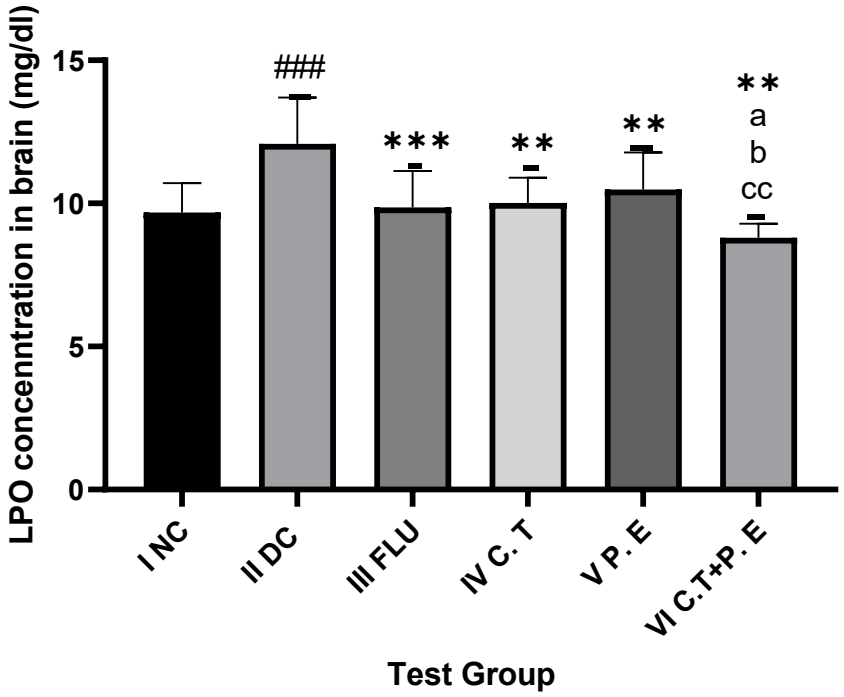

Figure no 6: Non-enzymic antioxidant Lipid Peroxidation (LPO) level in brain of mice

NC - Normal control, DC - Disease control, FLU – Fluoxetine 20mg/kg, C. T – Clitoria Ternatea 50mg/kg, P.E – Phyllanthus Emblica 2mg/kg, and C. T+P.E - Clitoria Ternatea 50mg/kg with Phyllanthus Emblica 2mg/kg.

Data are presented as means ± SD (n=6/group). One-Way ANNOVA followed by Bonferroni test for multiple comparison;

$^{\#\#}p<0.01$when compared to normal control (NC); $^{***}p<0.001$ when compared to disease control (DC); $^{a}p<0.05$ when compared to fluoxetine (III-FLU); $^{b}p<0.05$ when compared to Clitoria Ternatea (IV-C. T); $^{cc}p<0.01$ when compared to Phyllanthus Emblica (V- P.E).

4. Discussion:

Currently, depression is the most prevalent mental disorder across the globe and has evidenced an upward trend during the last few years. There are several treatment options for depression, including psychotherapy & medication. However, there are numerous disadvantages to using medications for depression, including resistance, relapse, late beginning of therapeutic efficacy, and adverse reactions [22]. As a result, there is a constant search for newer, more effective depression medications. Hence, plants have significantly been used in the ancient world as the cure for diseases, and many modern-day medications originate from plants [2].

The experiments were done to determine the efficacy of an aqueous extract of the leaves of Clitoria Ternatea & fruits of Phyllanthus Emblica for antidepressive action were carried out in Shock indeced mice models by using Actophotometer for the determination of locomotor activity

and identification of depressed mice, tail suspension experiment, and forced swimming experiment for immobility test. These are the tests that are most commonly applied to evaluate antidepressant drugs within the framework of the above-described behavioral models. Any drug or any extract which brings down the time of immobilization in these tests Is expected possess antidepressants [23].

In clinical practice, current pharmaceutical approaches to depression therapy have not yet produced the desired outcomes. There are still several first-line and novel therapies that seem to belong in the current drug classifications that have adverse effects [4]. There were several prospects for the creation of antidepressant medications from medicinal plants. Certain herbal or medicinal substances don't work well on their own or may only have a tiny impact, but when used in conjunction with other herbal medications, they have a synergistic antidepressant effect [24]. The increasing recognition of herbal medicines as effective treatments for mental health issues might be attributed to their improved quality. The development of phytomedicine with anti-anxiety and depressive properties still requires standardizing of extract and plant isolates, sufficient scientific evidence on the efficacy and safety protection of the variety of medicinal plants, and the establishment of suitable laws and regulatory bodies [25].

4.1. Behavioral Study:

This After shock induced depression in mice, the method for determining the depression in mice and is done by the actophotometer. Compared to other behavioral tests administered simultaneously, it presents accurate, quantifiable results to the central and peripheral motor activity that may offer comprehensive overview of the depression [26]. One of the most accurate means for evaluating the efficacy of antidepressant drugs in the treatment of depression is the forced swim test. The drugs are believed to affect the biosynthesis of neurotransmitters and thus have an antidepressant effect on the organism in case they reduce immobility time [27]. This particular test is standardized and this means that from across the different research and labs, the result is reliable and deferred across the different research and labs [28]. The contribution of monoaminergic systems (DA, NE, 5-HT) to the model of depression & antidepressant activity has been explored with forced swim test (FST) [29]. The majority of therapeutically useful antidepressants reduce the duration of the test's immobility phase and increase the frequency of fighting behavior. Another popular behavioral test used in preclinical studies to assess the effectiveness of antidepressant medications in mice is the Tail Suspension Experiment (TST) [30]. Similar to the Forced Swim Experiment (FST), this test is predicated on creating a state that is behavioral despair. Its importance comes from its capacity to yield accurate and quantitative information on the antidepressant efficacy of various substances [31]. This is a thorough explanation of the TST's importance in studies on antidepressant activity. A mouse is suspended by its tail during the TST, which causes it to become immobile. The rodent will struggle at first and try to get away, but eventually it will become immobilized from numerous failed attempts [32]. Effective antidepressants can lessen behavior despair, which is measured by how long this immobility lasts. When it comes to detecting substances with antidepressant qualities, the TST has strong predictive validity. Clinically successful depression medications typically shorten the TST's immobility period. Because the TST is a well-recognized and standardized approach,

findings may be consistently and reliably obtained in various lab settings and investigations [33]. The TST is an effective method for assessing a wide range of possible antidepressant chemicals, including synthetic and natural medications, because it is appropriate for high-throughput screening. The tail suspension test (TST) offers further information that augments the comprehension of a substance's antidepressant effectiveness when it is employed in conjunction with other behavioral tests like the FST. The TST provides a behavioral readout that corresponds with clinical results and is used to evaluate the effectiveness of both novel and established antidepressants [34].

4.2. Corticosterone level:

Corticosterone is a key biomarker for stress in mice, and levels of it indicate stress as well as depressive-like states in mice. A useful model for human depression is provided by the elevated corticosterone levels seen in numerous chronic stress paradigms that are utilized to elicit depressive-like behaviors in mice [16]. Increased corticosterone has a deleterious effect on synaptic plasticity and neurogenesis, especially in the brain's a portion of the brain linked to mood control. Hippocampal neurogenesis and synaptic plasticity can be enhanced by antidepressant medications that decrease corticosterone levels, offering new insights into the processes behind their therapeutic benefits [17].

4.3. Reduced Glutathion (GSH):

In the brain, glutathione (GSH) is a vital antioxidant that shields cells from oxidative damage. Reduced glutathione levels in depression models signify elevated oxidative stress, a condition which is connected to neuronal injury and dysfunction essential elements in the pathogenesis of depression [35]. Decreased glutathione levels are linked to depression via impairing mitochondrial activity and increasing apoptosis. Normalizing GSH levels in antidepressants can enhance the function of mitochondria and decrease apoptosis, offering new perspectives on the processes behind their therapeutic benefits [36].

GSH levels are measured in order to assess the effectiveness of antidepressant therapies. Antidepressants that are effective in lowering oxidative stress and enhancing neuronal function frequently restore GSH levels, and this restoration is linked to changes in behaviors similar to depression in mice [37].

4.4. Lipid peroxidation level:

One important marker of oxidative stress is lipid peroxidation (LPO), which is the result of free radicals breaking down lipids. In depression models, elevated LPO levels are frequently seen, indicating elevated oxidative damage, which is a key component of the patho-physiology of depression and causes damage to neurons [38].

Assessing LPO levels is crucial for determining how well anti-depressant therapies work. LPO levels are frequently lowered by effective antidepressants, suggesting a decrease in oxidative stress & an increase in cellular integrity. Improvements in mice's depressive-like behaviors are linked to this decrease [39]. Increased levels of lipoprotein (LPO) cause cellular damage, including deterioration of membrane integrity & functioning, and this can make depression symptoms worse. Lowering LPO levels in antidepressants helps prevent such cellular damage,

offering physiological understanding into their potential therapeutic benefits [40].

The use of particular models of animals in depression study has been validated by high levels of LPO. Preclinical studies have more translational relevance when models exhibit elevated LPO levels and concomitant depressive-like behaviors, since they are thought to be more representative of human depression [41].

5. Conclusion

A research animal model was used to assess the synergistic antidepressant impact of and Clitoria Ternatea & Phyllanthus Emblica in order to investigate their potential to jointly alleviate depressive-like behaviors. The possibility for creating novel, all-natural antidepressant medicines is shown by the synergistic effects seen when Clitoria Ternatea and Phyllanthus Emblica are used together. The results encourage more research on these medicinal plants' individual and combined pharmacological characteristics and therapeutic uses.

When Clitoria Ternatea and Phyllanthus Emblica were administered together, the antidepressant effectiveness was much higher than when each plant was administered separately. This study demonstrates a synergistic relationship between them that enhances their therapeutic benefits. The combined extract-treated shock induced depression mice had a significant decrease in depressive-like behaviors, as demonstrated by their better performance in behavioral tests including the tail suspension test (TST) and forced swim test (FST). These changes in behavior suggest that depressed symptoms have been effectively reduced.

In summary, Clitoria Ternatea and Phyllanthus Emblica together present a viable natural therapy option for depression with improved effectiveness and a good safety record.

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