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STANDARDISATION AND PHARMACOLOGICAL EVALUATION OF BRACLITAB SUSPENSION IN THE CHICK USING ANXIETY DEPRESSION MODEL

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ABSTRACT

Introduction: Depressive disorder is a prevalent psychiatric disorder, which affects 3.5 % of the world's population. The presently using drugs can impose a variety of side effects including cardiac toxicity, hypokinesia, sexual dysfunction, body weight gain, and sleep disorder. During the last decade, there is a growing interest in the therapeutic effects of natural products on mental disorders. Tabernaemontana divaritica, Clitoria ternatea, and Centella asiatica were investigated for antidepressant activity.

Methods: Braclitab suspension was prepared using the hydroalcoholic extract of Tabernaemontana divaritica, Clitoria ternatea, and Centella asiatica and then subjected to standardization followed by FTIR analysis to confirm herb-herb interaction. The antidepressant activity was studied through stress induction in the chick model and hydroalcoholic extract of was subjected to anti-depression activity in the chick model using stress vocalization and glutathione reductase activity using the chick model.

Results: It has been observed from our study that vocalization study is reduced with the drug administration and recent studies suggest that oxidative stress pathways may contribute to the pathogenesis of depression by interacting with the monoamine reuptake process. It was reported that individuals who suffer from depression displayed lower antioxidant potentials and reduced brain GSH levels by glutathione reductase assay.

Conclusion: However further study is needed to understand the mechanism of action and to identify the active component responsible for the antidepressant-like activity.

Keywords: Antidepressant Activity, GSH, Glutathione reductase, stress vocalization

INTRODUCTION

Global burden of disease – depression is the 2nd largest contributor in the year 2020 which affects 3.5% worldwide according to World Health Organization (WHO) [1]. This affects thought, behavior, sense, and feeling of well-being. Also, this imparts a loss of interest or pleasure in one's life summoned as anhedonia [2]. The major causes of depression are monoamine depletion like noradrenaline, serotonin, oxidative stress, overactivity of the HPA-axis and monoamine oxidase activates dopamine among depression the major cause is psychiatric illness [3].

In psychiatric disorders stress has been marked to play a significant role and it is also associated with suicide. And it is the most prevalent mental disorder was characterized by the loss of energy, profound feelings of gloominess, apathy, retardation of thinking and activity, despair and suicidal ideation, pessimism, slow thinking, and lack of initiative [4]. For the augmentation of powerful remedies, laboratory animals are essential. Bunny and Mc Kinney developed some of the most extensively cited prototypes [5].

ANTIDEPRESSANT IN CHICK MODEL

A beneficial adjuvant to conventional models may be the chick anxiety depression model, which stimulates all psychiatric syndromes within a single framework. The approach includes the stress of social separation that originally produces high vocalization of distress (DVoc) rates characteristic of an anxiety-like condition and that is followed by lower DVoc rates characteristic of state-like depression. Both stages can be dissociated pharmacologically such that different compounds with anxiolytic effects (e.g., clonidine) attenuate the high DVoc rates during the anxiety-like process while having antidepressant effects (e.g. fluoxetine) attenuate the decrease in DVoc rates during the depression-like phase [5].

METHODS

Subjects and housing characteristics

Cockerels (Gallus Gallus) were collected in a 1-day post-hatch and housed in stainless steel cages of 34x 57x40cm. Each cage contains 4 cages with 5 chicks and a total of 20 chicks. To alleviate the stress associated with experimenters, chicks are removed and briefly treated every day. Food and water are accessible from one quart of gravity-fed feeders and waters-ad libitum.

A room temperature of 29±1C and ov was

maintained.

Stress vocalization

Procedure

For the collection of isolation-induced stress vocalization, a six-unit test apparatus containing Plexiglas viewing chambers housed in sound-attenuating enclosures was used. Using 25W light bulbs, the units were illuminated and ventilated by an 8cm rotary fan in diameter. Miniature video cameras mounted in the corner of the enclosures at ground level and routes through the multiplexer allowed animal observation. Distress vocalization was gathered on the rear wall of the Plexiglas chamber via microphones, Lafayette instruments, etc., routed via sound triggering relays, and collected a USB interface via customized USB interface Software Developed.

Totally, 15 chicks aged 4 to 5 days were selected and divided into three groups of six in each group/ Only saline was administered in one group, and the suspension of the dose was administered in the second and third groups respectively. As low and high doses were administered by subcutaneous route respectively for one week and on the last day, after one hour of drug administration, the chicks of each group were placed in a plexiglass chamber and noted for microphone vocalization for a duration of 120 seconds.

Generally, the volume of sound would be lower in depressive condition chicks, and the antidepressant drug has the potential to improvise vocalization.

GSH ASSAY

ASSAY PROCEDURE

1. Prepare enough reaction mix for the standard and samples to be assayed in test tubes.

   Each well should contain:

   - 10 ml NADPH Generating mix
   - 10 ml Glutathione Reductase
   - 110 ml Glutathione Reaction Buffer

2. Mix well. Add 120 ml of the reaction mix to each well and incubate at room temperature for 10 minutes to generate NADPH.

3. Add 20ml of either the GSH standard solution or the sample solution. Incubate the plate at room temperature for 5-10 minutes.

4. Add 20ml of substrate solution and incubate at room temperature for 5-10 minutes or longer if the samples contain low levels of glutathione.

5. Read the absorbance at 405nm or 415nm using a microplate reader.

6. Determine the concentration of GSH in the sample solutions using the standard glutathione calibration curve.

RESULTS

The percentage yield of Centella asiatica

Table 1: Percentage yield of the three extracts

SI. No	Extract	Color	Percentage yield (w/w)
1.	Hydroalcoholic extract of Centella asiatica	Greenish yellow	3.70
2	Hydroalcoholic extract of Tabernaemontana divaricata	Dark green	2.88
3	Hydroalcoholic extract of Clitoria ternata	Brownish green	`1.64

Table 2: Phytochemical report of Centella asiatica, Tabernemontana divaricata and Clitoria terneta

SI.NO	CHEMICAL CONSTITUENTS	Ethanolic extract of Centella asiatica	Ethanolic extract of Tabernaemontana divaricata	Ethanolic extract of Clitoria ternata
1	Test for alkaloids	+	+	+
2	Test for reducing sugar	+	_	+
3	Test for proteins	+	+	+
4	Test for steroids	+	+	+
5	Test for phenols	-	+	+
6	Test for tannins	-	+	+
7	Test for flavonoids	+	+	+
8	Test for gums and mucilages	_	+	_
9	Test for glycosides	+	+	+
10	Test for saponins	_	+	+
11	Test for terpenes	_	+	+

Table 3: Standardization of Suspension

S. No	Groupings	Sound recorder(db)
1	Control	27.8+2.90
2	Low dose suspension(10mg)	42.6+2.05***
3	High dose suspension(20mg)	62.08 + 2.12***

Table 4

Formulation	Redispersibility	pH	Flow rate	Sedimentation Volume	Viscosity	Crystal growth
Low dose	Good	7.2	5ml /48sec	1.23		-
High dose	Good	7.3	5ml/50sec	1.24		

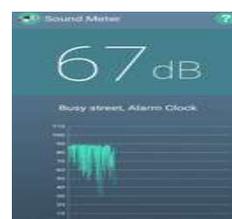Fig 1.1 Positive control   Fig1.2 Low dose suspension   Fig 1.3 High dose suspension
Figure 1: Representation of voice recorder in distress vocalization study
All the groups were statistically proved by students' T-test and the values were expressed by Mean $\pm$ SEM, P***<0.001

Table 5: Study of glutathione reductase in the anti-depressant study

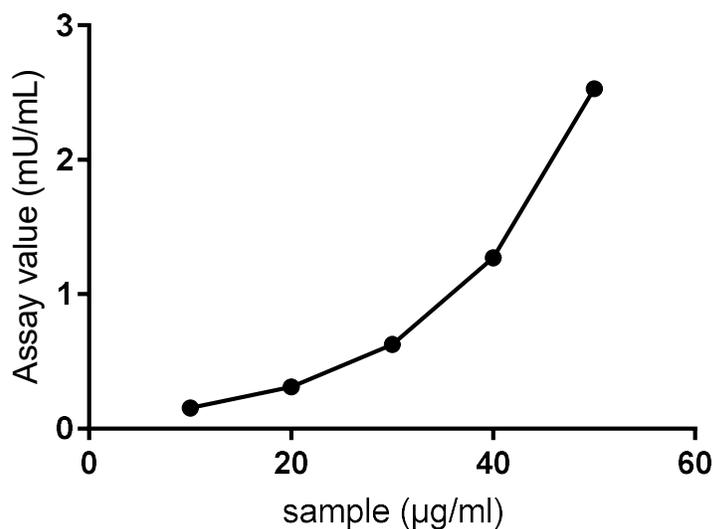| Treatment	Optical density (405nm)
STANDARD 10 (µg/ml)	0
STANDARD 20 (µg/ml)	0.31
STANDARD 30 (µg/ml)	0.62
STANDARD 40 (µg/ml)	1.27
STANDARD 50 (µg/ml)	2.53
POSITIVE CONTROL	0.24
LOW DOSE (1ml)	0.49
HIGH DOSE(1ml)	0.63

Figure 2: Standard graph of glutathione reductase

FTIR PEAK VALUES FOR CLITOREA TERNATEA HYDROALCOHOLIC LEAF EXTRACT

SI.NO	PEAK VALUES	FUNCTIONAL GROUPS
1	3389.57	Alcohols
2	2925.41	Alkanes
3	2856.66	Alkanes
4	1632.33	Primary amines
5	1409.06	Aromatic amines
6	1057.61	Carboxylic acids
7	926.50	Alkene
8	869.00	Primary

FTIR PEAK VALUES FOR CENTELLA ASIATICA HYDROALCOHOLIC LEAF EXTRACT

SI. NO	PEAK VALUES	FUNCTIONAL GROUPS
1	3425.82	Alcohol
2	2922.15	Aliphatic compounds
3	2853.49	Aldehyde
4	1736.26	Mostly aldehyde or ketone
5	1629.22	Amide
6	1437.36	-C=C
7	1322.00	Aromatic amines
8	1055.34	Aryl/venyl ether
9	893.13	Aromatic –C-H
10	622.41	Aromatic halides

FTIR PEAK VALUES FOR TABERNAEMONTANA DIVARITICA HYDROALCOHOLIC LEAVES EXTRACT

SI NO	PEAK VALUES	FUNCTIONAL GROUPS
1	3400.00	Alcohol
2	2800.23	Carboxyl
3	1650.43	Aldehydes or ketones
4	1437.11	-C=C
5	1225.00	Aromatic C-H
6	800.26	Alkene
7	650.38	Aromatic C=C

DISCUSSION

Depression is a mood disorder that has a high prevalence in women and is also a heritable disease. Depressive disorder is a prevalent psychiatric disorder, which affects 21% of the world's population. The presently using drugs can impose a variety of side effects including cardiac toxicity, hypoplasia, sexual dysfunction, body weight gain, and sleep disorder. During the last decade, there is a growing interest in the therapeutic effects of natural products on mental disorders.

The leaves of Centella asiatica, Tabernaemontana divaricata, and Clitoria ternata were collected from the Kancheepuram district, Tamil Nadu for the proposed study and were authenticated by Prof. P.Jayaraman, Director; Plant anatomy research Centre (PARC), Medicinal plant research unit, West Tambaram, Chennai, and a specimen was deposited. All three plants were individually coarsely powdered and extracted with 70% ethanol and the yield was tabulated in **Table 1**. The phytochemical analysis revealed the presence of alkaloids, flavonoids, proteins, saponins, terpenoids, and steroids, as mentioned in **Table 2**.

The low dose and high dose of suspension were standardized by the evaluation of pH, viscosity, redispersability, and flow rate. Both the suspensions were free from crystal growth.

Pharmacological activity

1. **Sound vocalization assay**

Chicks are precise birds; they are very active in their environment from the hatch. It has large size and well-developed brains which have fine soft unossified skulls. The training session of the young chick includes the specific task suitable for the particular species and which is significant event in the life of the young chick and it should be readily measurable brain changes of the chick [6, 7].

In this study, eight days old chick produced the least vocalization as an index of depression, due to its isolation from the mother. In the suspension-treated group, there is an increase in vocalization due to a decrease in depression and the data were

mentioned in **Table 4**. The dependent measures were distress vocalizations to index separation stress **[8, 9]**. The suspension reverses the distress of vocalization in a dose-dependent manner.

2. Glutathione reductase assay

Recent studies suggest that oxidative and nitrosative stress pathways may contribute to the pathogenesis of depression by interacting with neurogenesis/neuroplasticity, neuroinflammation, and the monoamine reuptake process **[10, 11]**. The current thought is that antidepressants exert their therapeutic effect by suppressing proinflammatory cytokines and ROS/RNS production or enhancing anti-oxidant defense. Molecular targets involved in the oxidative/nitrosative stress including nicotinamide adenine dinucleotide, niacin, brain-derived neurotrophic factor, glycogen synthase kinase 3, heme oxygenase, nuclear factor E2-related factor 2, and telomerase are believed to be connected to the pathophysiology of depression **[12]**.

It was reported that individuals who suffer from depression displayed lower serum/plasma antioxidant potentials and reduced brain GSH levels. Also, F2-isoprostanes circulatory levels are increased in depressed subjects and are correlated with the severity of depressive symptoms. Urinary excretion of 8-hydroxydeoxyguanosine [8-OHdG] seems to be higher in depressed patients when compared to healthy controls **[13]**.

The interpolation of the graph was done manually for the low dose and high dose suspension optical density was plotted against the concentration of standard and the suspension optical density was intercepted. The concentration of the total glutathione reductase of low-dose suspension was found to be 0.63mu/ml and for high-dose suspension was. 1.65mu/ ml. The suspension increases the effectively reverses the total GSH in a dose-dependent manner.

SUMMARY & CONCLUSION

Depression is a potentially life-threatening disorder that affects hundreds of millions of people all over the world. It can occur at any age from childhood to late life and is a tremendous cost to society as this disorder causes severe distress and disruption of life and, if left untreated, can be fatal. The psychopathological state involves a triad of symptoms with low or depressed mood, anhedonia, and low energy or fatigue. Other symptoms, such as sleep and psychomotor disturbances, feelings of guilt, low self-esteem, suicidal tendencies, as well as autonomic and gastrointestinal disturbances, are also often present.

In this study, the three plants namely, Centella asiatica, Tabernaemontana divaritica and Clitoria ternatea were selected and extracted with 70% alcohol by

maceration, followed by phytochemical screening and then with standard procedure formulation of low dose and high dose suspension using three extracts were performed.

The two suspensions were standardized by reviewing their PH, viscosity, flow rate, and redispersibility and there was no crystal growth in the two formulations and was focused to pharmacological screening.

In the pharmacological studies, the novel chick model was performed, in which distress vocalization was measured using a sound recorder, compared to the positive control group, the low-dose and high-dose treated groups improved the vocalization thereby reversing the depression.

In the glutathione assay, the biomarker enzyme in oxidative stress is decreased in the depression group but it has reversed in low-dose and high-dose suspension groups indicating that the drug suppresses depression and promotes CNS stimulation.

Finally, the extract was subjected to FT-IR for spectral analysis of functional groups present in three plants. The results suggested that all have aromatic amines, alkenes, and aliphatic compounds which have an impact on the pharmacological activity.

Further study is essential to focus this suspension in clinical trials in order to combat depression in this busy schedule society and to promote a healthy lifestyle.

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