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EVALUATION OF ANTI DEPRESSION ACTIVITY OF POLYHERBAL FORMULATION IN RESERPINE INDUCED DEPRESSED ANIMAL MODEL

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Abstract:

The present study was designed to evaluate the anti depression activity of methanolic extract of shankhapushpi, alpinia officinarum and ginkgo biloba against reserpine induced depressed animal model. The study was carried for 21 days. Depression disease was induced using reserpine 0.75mg/kg b.w. The phytochemical screening was carried out and showed the presences of flavonoids, alkaloids, phenolic compounds, steroids, terpinoids, and glycosides. Swiss albino mice of 25-30grams were used for the study. Depression disease was screened by absorving behavioural parameters using method force swim test and tail suspension test and also evaluating the biochemical parameters like estimation of dopamine , serotonin and nor epinephrine. The treatment with methanolic extract of shankhapushpi, alpinia officinarum and ginkgo biloba(MESAG) 250mg/kg and 500mg/kg effectively decreases the immobility time in Force swim test(FST) and Tail suspension test(TST) when compared with the disease control group and also enhances the levels of serotonin ,nor epinephrine and dopamine in treated group when compare with the disease control. The MESAG treated groups showed similar results to standard group. Thus the results suggested that MESAG posses anti depression properties.

Keywords: *Depression disease, Reserpine, Clomipramine, Methanolic extract of shankhapushpi(Convolvulus pluricaulis), Alpinia officinarum, Ginkgo biloba. Serotonin , nor epinephrine and dopamine.*

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INTRODUCTION:

Psychiatric diseases are the range of conditions which affects neurons, neurotransmitters and brain. This diseases are incurable and debilitating conditions that result in progressive degeneration or death of nerve cells, which causes problems with movement or mental functioning.[1] Depression is common, recurrent, chronic, disorder which affects negatively about how you feel, think and behave. It can lead to variety of physical and emotional imbalance that affects the person ability. [2] Depression mainly develops when there are defects in the monoaminergic neurotransmitter system. Changes in the serotonin, nor epinephrine and to some extent dopamine leads to development of depression. Some medical conditions like thyroid, vitamin deficiency and brain tumor can also mimic like symptoms. So it is very important to rule out the exact cause of it. It can occur across the entire life cycle and is the most common of the severe psychiatric illnesses. If it is left untreated then it affects other systems of the body. The diagnostic and statistical manual of mental disorder has reported a list of symptoms for depression [3] includes irritability, anxiety, hopelessness, suicidal ideation, depressed mood. An individual should experience at least five or more symptoms for at least 2 weeks. Researches in 2017 revealed that one in five people suffering with coronary disease and heart stroke has depression. The World Health Organization predicts that by 2020, major depression will become the second leading cause of worldwide disease burden, surpassed by Ischemic heart disease. [4]

The plants extracts have always been good source of treatment in many diseases, like wise the present therapy choses to poly herbal formulation was used for the treatment of depression disease. The poly herbal formulation comprises of three plants namely shankhapushpi(convolvulus pluricaulis) leaves belonging to the family Gentianaceae which is found in India and Burma mostly used in ayurveda because of its tremendous benefits. In English it is commonly called as aloe weed [5].All parts of the plant are loaded with therapeutic benefits. These plants have the ability to power up the brain and enhance brain function. The other plant is alpinia officinarum rhizomes belonging to the family zingiberaceae and is commonly called as lesser galangal which is used in thai cooking because of its strong aroma and spicy flavor. [6] Mostly it is cultivated in south Asia and its origin is china. It has anti inflammatory, anti-fungal, carminative,anti tumor, hypothermia, anti-diuretic, analgesic activity, immunomodilator, anti-viral activity, anti helminthic activity, neuro protective, anti malarial activity. The last plant is ginkgo biloba leaves belonging to the family Ginkgoaceae. Gingko biloba which is a native of china which is cultured for last thousands of years and is the Chinese rich heritage. It has many medical benefits it is mainly used to treat tinnitus, dementia, anxiety, vertigo, Alzheimer's, circulatory disorders, depression and cerebral dysfunction. [7]

Depression is not a preventable disease but maintaining diet and exercise can prevent the progression of the disease. The treatment includes selective serotonin reuptake inhibitors [SSRI] like citalopram, fluoxetine and sertraline at a dose of 20mg per day.It acts by inhibiting the reuptake of serotonin in synaptic vesicles. Selegiline (Emsam), which is a new MAOI that sticks on the skin as a patch, and leads to fewer side effects than other MAOIs. These medications should not take in combined with any SSRIs. When the drug treatment is not effective other therapies like ECT and TMS is performed.

MATERIAL AND METHODS:

Animals:

Swiss albino mice strain (25-30g) were used for the study.The experimental mice were procured from Sainath Animal Agency, Musheerabad, Hyderabad, India. The mice were kept under standard well controlled conditions before and throughout the experimental duration. The temperature was maintained at 22°C (±3°C) and relative humidity was between 50-60%. The animals were given pellet diet and drinking water ad libitum, kept in 12h/h light/dark cycle and maintained for atleast 5 days prior to dosing to allow for acclimatization to laboratory conditions. The experimental protocol was given approval by Institutional Animal Ethical Committe and was carried under the compliance of IAEC guidelines. (IAEC/SUCP/2019/08).

Plant material collection and extraction:

The Plants parts of *shankhapushpi leaves ,alpinia officinarum rhizomes and leaves of ginkgo biloba* were collected from, identified and authenfied by Dr. Ghousia tabassum: Hyderabad Unani Research Foundation. Hyderabad, Telangana State. The plants were cleaned , shade dried, coarsely powdered and sieved through sieve no.100. The methanolic extract of *shankhapushpi ,alpinia officinarum and ginkgo biloba was prepared.* This powder was used for solvent extraction. The MESAG was formulated in 2:2:1ratio(*Shankhapushpi, alpinia officinarum, ginkgo biloba*)About 250gms of powder poly herbal powder extract was subjected to soxhlet extraction using 500ml solvent ethanol. This cycle was repeated many times until the colour of the solvent in the siphon of the soxhlet faded away. The extract was

concentrated on water bath. The MESAG was suspended in normal saline and administered orally.

Phytochemical screening:
The MESAG was subjected to various phytochemical tests to identify chemical constituents such as flavanoids, alkaloids, glycosides, phenolic compounds, steroids, terpinoids, amino acids, carbohydrates, etc.[8]

Reserpine induce depression:
Reserpine is an alkaloid extracted from the roots of an Indian plant Rauwolfia serpentine which is also called as sarpagandha, belonging to the family Apocynaceae. It is an anti hypertensive drug used to treat mild hypertension. But because of its tremendous side effects it is not in use currently. Most common side effects of Rauwolfia are cancer, depression sedation, nausea, vomiting etc. It induces depression by depletion of monoamines such as dopamine, adrenaline, serotonin, nor epinephrine in the brain after its prolonged use.[9]The mechanism by which it induce depression is, it binds irreversibly to the storage vesicles of monoaminergic neurons and inhibits the release of the neurotransmitters in the synapse after depolarization. The neurotransmitters in the storage vesicles leak in to the cytoplasm where it is destroyed by the intraneuronal monoamine oxidase leading to the depletion of neurotransmitters.
Depression was induced in the given test animals by administration of reserpine orally at a dose of 0.75mg/kg body weight of the animal. About one gram of Rauwolfia powder consists of 33mg of reserpine.

Experimental design:
Thirty animals were divided in to five groups comprising of six animals in each group
30 minutes before administration of test and standard drug, reserpine 0.75mg/kg b.w was administered orally by dissolving in 0.9% of normal saline.

- **Group 1** : Normal control
  Served as control and received oral administration of 0.9%NACL at a dose 1ml/kg body weight p.o once a day for a duration of 21days.
- **Group 2**:Disease control
  This group was administered reserpine 0.75mg/kg body weight i.p for a duration of 21days.
- **Group 3**:Standard
  This group was given clomipramine 25mg/kg bodyweight i.p and reserpine 0.75mg/kg body weight orally .
- **Group 4** : Methanolic extract 1 dose 250mg/kg body weight orally + Reserpine 0.75mg/kg body weight orally.
- **Group 5** : Methanolic extract 2 dose 500mg/kg body weight orally + Reserpine 0.75mg/kg body weight orally.

Behavioural screening studies:
Force swim test: This test was developed by porsolt et al [10] in the year 1978 and it was the one of the tests which measures the behavioral assessments in animals. This test was conducted in a cylinder container which is 18cm in diameter and 15cm in height. Water was poured in to the cylinder where the temperature was maintained at 25c.Mice was placed in the cylinder container and allowed to swim in the water. After the continues struggle each mice assumes an typical immobile posture. Mice was consider as immobile when it was making minimal movements of limbs just to keep its head above the water and floating in the water without any struggle. This test is conducted for 5 min and total duration of immobility was measured per second. Changes in the immobility time was recorded after the administration of drug.

Tail suspension test: TST is the another method to test the depressive like behavior in the animal.It was performed according to the method described by steru et al.[11] In this method the animal is allowed to suspend on the edge of the shelf 50cm above the ground/table by using adhesive tape placed above 1cm from the tip of the tail. It was conducted for 6 min where the duration of immobility was recorded per second.[12] Mice was consider as immobile and depressed when it was completely motionless.

Dissection and Homogenisation:
On 21st day, the experimental mice were sacrificed by giving overdose of anaesthetic ether. Biochemical estimation was performed after the completion of 21 days, where the animals were sacrificed by using overdose of ether. After the death of animal, brain was dissected out and placed in ice cold phosphate buffer PH 7.Then it was weighed and used for estimation of dopamine ,serotonin and nor adrenaline by habibur rahmaan and Schlumpf et al method. After the braine dissection wet weight of the braine tissue was measured. Then the braine was homoginated in a homogination tube which consists of HCL-butanol in a ratio of (1:10) and homogination was carried out for 1 min. After the homogination the brain was centrifuged at 2000rpm for about 20 min. About 0.8ml of the supernatant phase was taken from the centrifuge tube and to it about 0.25ml of 0.1M Hcl and 2ml of hexane were added. The upper organic phase was discarded and the lower aqueous phase was used for the estimation of neurotransmitters.

Preparation of buffers:

- **0.4M HCL**: 3.4ml concentrated HCL and volume was made up to 100ml with water.
- **Sodium acetate buffer (PH 6.9)**: To prepare sodium acetate buffer Acetic acid 1M was prepared(by adding 5.7 ml of glacial acetic acid and volume was made up to 100ml with the help of water). About 2.8ml of this was taken. To the 1M acetic acid 27.33ml of 0.3M Sodium acetate (prepared by adding 4.08g of sodium acetate and dissolved in 100ml distilled water) was added and the volume was made up to 100ml by adding water and sodium hydroxide solution was added accordingly to adjust PH.
- **5M NAOH**: sodium hydroxide pellets about 20 g was dissolves' in 100ml of distilled water.
- **0.1M Iodine solution** : Potassium iodide about 4g and Iodine about 2.6g was taken accurately and dissolved in 100ml of ethanol
- **NA_2SO_3 Solution**: In 2ml of water 0.5g of Na_2SO_3 was added. To it 18ml of 5M NaoH was added.
- **10M Acetic acid**: Glacial acetic acid 57ml was dissolved in 100ml distilled water
- **Estimation of dopamine:**
- To estimate the level of dopamine, on 21st day the animal was sacrificed and braine was dissected to estimate the level of neurotransmitters by fallowing the method described by habibur rahmaan.[13]

Reagents:

- 0.4M HCL
- 0.1M HCL
- Sodium acetate buffer
- Hexane
- Iodine solution0.1M
- Butanol
- Sodium sulphite
- Acetic acid

Procedure:

About 0.2ml of aqueous layer was taken for the estimation of dopamine. To that aqueous phase chemicals like 0.05ml of 0.4M Hcl is added and 0.1ml of sodium acetate buffer along with 0.1ml of iodine solution were added for the oxidation purpose. This process was continued after 2 min by addition of 0.1ml of sodium sulphite solution. After 1.5min 0.1ml acetic acid is added and the solution is heated at 100c for 6min.then it is cooled down at room temperature .AT 330-375nm emission and excitation is measured at spectrofluorimeter.

Estimation of Nor adrenaline:

Reagents:

- 0.4M HCL
- 0.1M HCL
- Sodium acetate buffer
- Hexane
- 5M NAOH
- Na_2So_4 Solution.
- Iodine solution0.1M
- Butanol
- Acetic acid

Procedure:

About 0.2ml of aqueous layer was taken for the estimation of Nor adrenaline. To that aqueous phase chemicals like 0.05ml of 0.4M Hcl is added and 0.1ml of sodium acetate buffer along with 0.1ml of iodine solution for the oxidation purpose. This process was continued after 2 min by addition of 0.1ml of Na2So4. After 1.5min 0.1ml acetic acid was added and the solution is heated at 100c for 6min.Then it was cooled down at room temperature .AT 395-485nm emission and excitation was measured using spectrofluorimeter.

RESULTS AND DISCUSSION:

Phytochemical screening

The preliminary phytochemical analysis of MESAG confirmed the presence of flavonoids, phenolic compounds, amino acids, steroids, tannins, saponins, terpenoids, amino acids, carbohydrates and alkaloids suggesting its potential antioxidant activity

Force swim test: Figure no:1

Effect of MESAG on immobility time was determined in of experimental mice. The values are expressed as Mean ± SEM (n = 6). One way ANOVA was carried out followed by dunnetts multiple comparison test.The value is expressed as$P < 0.001$.

Inference: The immobility time was evaluated by performing FST on 7th ,14th and 21st days respectively and found that the immobility time is reduced gradually in the MESAG treated groups when compared with the negative control. Standard group is compared with the MESAG treated groups and found the p value P<0.0001 which was significant.

Tail suspension test: Figure no:2

Effect of MESAG was determined by performing TST. The values are expressed as Mean ± SEM (n = 6). One way ANOVA was carried out followed by dunnetts multiple comparison test.The value is expressed as$P < 0.001$.

Inference: The immobility time was evaluated by performing TST on 7th ,14th and 21st days respectively

and found that the immobility time is reduced gradually in the MESAG treated groups when compared with the negative control. Standard group is compared with the MESAG treated groups and found the p value P<0.0001 which was significant.

Estimation of neurotransmitters: Figure no: 3 On 21st day of experiment,The neurotransmitter levels like dopamine, nor adrenaline and serotonin was evaluated by UV spectrophotometer.The observance value is calculated at specific wave length.

Effect of MESAG on the levels of neurotransmitters like serotonin, dopamine and nor adrenaline was determined on experimental mice. The values are expressed as Mean ± SEM (n = 6). One way ANOVA was carried out followed by dunnetts multiple comparison test.The value is expressed as$P < 0.001$.

Inference: The neurotransmitters levels, dopamine, serotonin, and nor epinephrine in MESAG treated groups was increased when compared with the negative disease control group. The standard group showed increased levels of neurotransmitters and was similar to that of MESAG treated groups. This reveals that MESAG posses anti depression activity.

Depression is common, recurrent, chronic, disorder which affects negatively about how you feel, think and behave. Depression is with the human kind since the beginning of recorded history. Melancholia was the name of depression which was given by Hippocrates, which means black bile. In 19th centaury depression is described as inherited temperament weakness. Depression has become huge public problem effecting millions of people around the globe. In the USA, the lifetime prevalence was 16.2% (32.6-35.1 million adults) and the 12-month prevalence was 6.6% (13.1-14.2 million adults).Various neuropsychiatric illness is associated with the deficiency or over abundance of neurotransmitters in certain parts of the braine The main hallmark of depression alteration in the levels of serotonin, nor adrenaline and to some extent dopamine. Despite of several research, it is still unknown whether low level of neurotransmitters causes depression or whether depression causes lower level of neurochemicals in the braine. In order to treat symptoms, mostly prescribed drugs are SSRIs. In case of major depression doctors prescribes combination of different antidepressants for symptomatic relief. In severe cases electro convulsive therapy and transcrainal magnetic stimulation are effective. All theses therapies may reduce the quality of life.

With the increase in prevalence of depression and with an increase in the adverse effects of present drugs, there has been a increase in research on plants and traditional treatments to identify new potential therapy options which can be used in the treatment of depression.It has been confirmed that oxidative stress may be involved in the development of depression. Hence plants which posses antioxidant activity may have the neuroprotective effect and can be applied in the treatment of depression. The phytochemical test of MESAG confirmed presence of flavonoids, alkaloids, polyphenols, triterpenes etc showing its potential antioxidant scavenging activity.

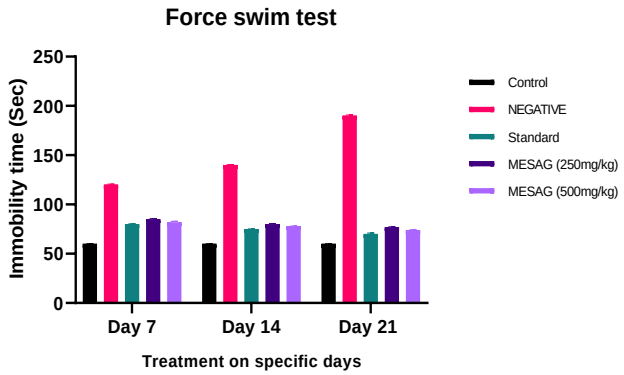
Reserpine induced depression animal model was used for this study, where evaluation of anti depression activity of polyherbal formulation in reserpine induced animal model (swiss albino mice)was done. In the present study reserpine (0.75mg/kg body weight i.p) administered for 21 days and found to induce depression by increasing the immobility time to a great extent since animals belonging to reserpine treated group (190±6.8) had greater immobility time in force swim test and (198±0.2) immobility time in tail suspension test on 21st day. Treatment with polyherbal formulation at (250mg/kg body weight) gave an immobility time (68±1.2) in force swim test and (113±0.3)in tail suspension test. Where as treatment with poly herbal formulation at (500mg/kg body weight) gave immobility time (66±0.7) in force swim test and (112±0.1) in tail suspension test on 21st day.This was found to be similar in clomipramine treated group (75±0.9) in FST and (110±0.4) in TST an decreased in immobility time.

Alteration in the levels of neurotransmitters in the braine is the underlying mechanism of depression. Hence neurotransmitters were evaluated on the 21st day of experiment, where the animals were sacrificed and mice brain homogenates were prepared. The mice brain homogenates were subjected to estimation of nor adrenaline , serotonin and dopamine levels.

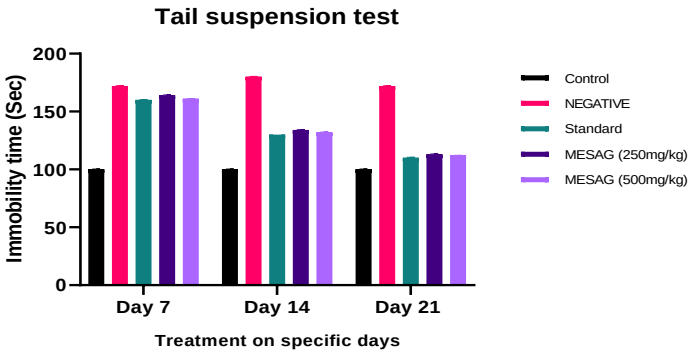
Dopamine also have a role to some extent in the depression where lower levels of dopamine is observed in the depressed persons. In the present study similar results were observed. In the reserpine treated groups the dopamine levels are (22.9±0.5) on 21st day. On treatment with polyherbal formulation at a dose (250mg/kg body weight) and (500mg/kg body weight) shows dopamine levels (24±0.4) and (25±0.1) respectively. This was similar to that of standard clomipramine treated group (26±0.2).

Serotonin is the key hallmark of depression where decreased levels of it develops the symptoms of depression. In the present study similar results were observed, where reserpine treated group shows lower levels of serotonin(32.3±0.5) on 21st day of treatment .On treatment with polyherbal formulation at a dose of (250mg/kg) and (500mg/kg) body weight shows increased serotonin levels(45±0.4) and (49±0.3) respectively. This was similar to that of standard treated group(50.8±0.2).

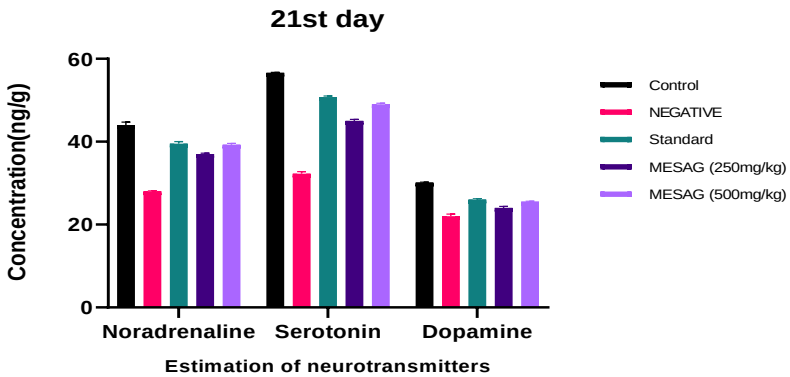
Nor adrenaline deficiency leads to the development of depression. It was observed in the present study that negative treated groups shows lower level of nor adrenaline (28±0.1) on 21st day .On treatment with the polyherbal formulation dose (250mg/kg) and (500mg/kg)body weight shows increased levels of nor adrenaline (37±0.2) and (39.3±0.3) respectively. This was similar to that of clomipramine treated group (39.5±o.5)



Force swim test: Figure no:1



Tail suspension test: Figure no:2



Estimation of neurotransmitters: Figure no: 3

CONCLUSION:

The methanolic extract of poly herbal extract containing leaves of ginkgo biloba , rhizomes of alpinia officinarum and leaves of shankhpushpi were selected to study the anti depression activity in reserpine induced depressed animal model.

The prospective anti depression activity of polyherbal extract could be inferred by the following results. Phytochemical analysis of the extract shows the presence of flavonoids, alkaloids, steroids ,phenols and terpinoids.

The behavioural studies of the mice treated with the polyherbal extract showed an decreased in the immobility time in comparison to reserpine treated groups. The present study also revealed the treatment with the polyherbal extract increased the levels of neurotransmitters like serotonin, nor adrenaline and dopamine in the braine of mice.

Thus it can be concluded that the polyherbal extract have a favourable anti depression activity and is potential low cost , potent herbal medicine for braine disorders like depression ,that is relatively safe than the conventional therapy. However the depth research on the anti depression and neuroprotective potential of poly herbal compound is required using more experimental paradigms and detailed methods to identify mechanism that could be invested for the prevention and treatment of depression disease.

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