ISSN:0975-1459
Journal of Pharmaceutical Sciences and Research
www.jpsr.pharmainfo.in

Preparation of Polyherbal Formulation for Antidepressant activity.

Bharat Kumar Tawani* and S Anitha

Department of Pharmacognosy, Government College of Pharmacy Bengaluru-560027 Karnataka, India

Abstract:

In Present modern life style depressant is the most frequent psychiatric condition in majority of population and this become important area of research in psychopharmacology so it is now contemporary to search some safe and effective alternative. Keeping this view in mind the present study was undertaken to investigate the anti-depressant activity of polyherbal formulation. Formulation consists of methanolic extract of *Ocimum sanctum, Centella asiatica, Curcuma longa, Withania somnifera, Emblica officinalis* all of which are classified in formulation which are reported to promote physical and mental health. The Polyherbal formulation was standardization by using various parameters. Evaluation of Antidepressant activity by using; Forced swim test. In this model the animals were divided into four groups in which first group receiving 1% acacia as control , second group receiving 5mg/kg diazepam as standard and further two group receiving Polyherbal formulation of 200mg/kg and 400mg/kg body weight of animals.

Key words: Diazepam, 1% acacia, polyherbal formulation, Anti-depressant activity.

INTRODUCTION:

Depression is a state of low mood and aversion to activity that can affect a person's thoughts, behavior, feelings and sense of well-being. Depression people feel sad, anxious, empty, hopeless, worried, irritable or restless. They may lose interest in activities that once were pleasurable, experience loss of appetite or overeating, have problem concentrating, remembering, details, or making decisions, and may contemplate, attempt, or commit suicide.

Depression mood is also a primary or associated feature of certain psychiatric syndromes such as clinical depression. In the medical terms we can say that depression is a prevalent psychiatric disorder with estimates reaching as high as 21% of the world population. Despite the fact that it is a psychiatric disorder, the world health organization predicts that it will be the second leading cause of death by the year 2020. An estimated 7-12% of men and 20-25% of women experience a depressive episode in their life time[1].

Mental depression is a chronic illness that affects a person's mood, thoughts, physical health and behavior. There are two types of mental depression, namely unipolar depression, in which mood swings are always in the same direction and is common(about 75% of case), non-familial, clearly associated with stressful life events, and accompanied by symptoms of anxiety and agitation. The second type is bipolar depression (about 25% of cases), sometimes also called as endogenous depression, shows a familial pattern, unrelated to external stresses and usually appears in early adult life, and is much less common, results in oscillating depression and mania over a period of a few weeks[2].

The polyherbal formulation consist of *Centella asiatica*, *Ocimum sanctum, Withania somnifera*, *Curcuma longa, Emblica officinalis.*

The Polyherbal formulation was standardized by using various parameters such as organoleptic evaluation, Physio-chemical investigation, Determination of pH, Fluorescence analysis, and Density, Visocity, Surface tension, Preliminary phytochemical screening. [3]

Even though many plants are available to treat depression, here an attempt is made to prepare, standardize and evaluate the polyherbal formulation for antidepressant activity.

MATERIALS AND METHODS:

I. Collection and authentication of Crude drug:

The plant materials of *Ocimum sanctum, Centella asiatica, Curcuma longa, Withania somnifera, Emblica officinalis* were collected from Amruth kesari depot and local market Bengaluru, India The authentication of the plant materials was done by Dr. V. Rama Rao, National Ayurveda Dietetics Research Institute Bengaluru, India. The respective part of plant material were dried in shed and made into coarse powder for extraction.

II. Extraction method:

The extraction of plant materials were carried out using soxhlet assembly *Centella asiatica* and *Ocimum sanctum* (Whole plant), *Emblica officinalis* (dried fruit), *Curcuma longa*, (dried rhizome), *Withania somnifera* (dried roots) for 2-3 days. The extracts were dried, weighed and stored in air tight bottles[4].

III. Preliminary Phytochemical Screening of Polyherbal formulation:

The polyherbal formulation were subjected to qualitative tests in order to identify class of compound like Carbohydrates, Proteins, Alkaloids, Glycosides, Tannins, Saponins, Flavanoids Fixed oils, etc [3].

IV. Standardization of Polyherbal formulation:

The various standardization parameters studied were organoleptic properties, Physico-chemical investigations, determination of pH, Fluorescence analysis, determination

of viscosity, surface tension, density, and Preliminary phytochemical analysis [3].

a) Organoleptic evaluation:

The Organoleptic evaluation refers to evaluation of the formulation by color, odor, taste[3].

b) Physico-chemical investigation:

Physico-chemical investigation of formulation includes Water soluble extractive value, Alcohol soluble extractive value, Pet ether soluble extractive value, Chloroform soluble extractive value, Total ash value, Water soluble ash, and Acid insoluble ash[3].

i) Determination of Water-soluble Extractive:

5 grams of air-dried plant material was macerated with 100 ml of water in a closed flask, with shaking frequently during the first 6 hours and allowed to stand for 18 hours separately. Thereafter, it was filtered rapidly taking precaution against loss of water. Evaporated 25ml of filtrate to dryness in a tared flat bottom shallow dish dried at 105^0C and weighed. Percentage water soluble extractive was calculated with reference to the air-dried drug[4].

ii) Determination of Ethanol-soluble Extractive:

5 grams of air-dried plant material were macerated with 100 ml of ethanol in a closed flask with shaking frequently during the first 6 hours and allowed to stand for 18 hours separately. Thereafter, it was filtered rapidly taking precaution against loss of ethanol. Evaporated 25ml of filtrate to dryness in a tarred flat bottom shallow dish dried at 105^0C and weighed. Percentage ethanol soluble extractive was calculated with reference to the air-dried drug[4].

iii) Determination of Chloroform-soluble Extractive:

5 grams of air-dried plant material were macerated with 100 ml of chloroform in a closed flask with shaking frequently during the first 6 hours and allowed to stand for 18 hours separately. Thereafter, it was filtered rapidly taking precaution against loss of chloroform. Evaporated 25ml of filtrate to dryness in a tarred flat bottom shallow dish dried at 105^0C and weighed. Percentage chloroform soluble extractive was calculated with reference to the air-dried drug[4].

iv) Determination of Pet ether-soluble Extractive:

5 grams of air-dried plant material were macerated with 100 ml of Pet ether in a closed flask with shaking frequently during the first 6 hours and allowed to stand for 18 hours separately. Thereafter, it was filtered rapidly taking precaution against loss of pet ether. Evaporated 25ml of filtrate to dryness in a tarred flat bottom shallow dish dried at 105^0C and weighed. Percentage pet ether soluble extractive was calculated with reference to the air-dried drug[4].

v) Determination of Total Ash:

2-3 grams of dried powder was accurately weighed crude drug powder in a tarred platinum or silica dish previously ignited and weighed. Scattered the powder drug on the bottom of the dish, incinerate by gradually increasing the heat not exceeding dull red heat until free from carbon, cool and weight. If a carbon free ash cannot be obtained in this way, exhaust the charred mass with hot water, collect the residue on an ash less filter paper, incinerate the residue and filter paper, add the filtrate, evaporate to dryness and ignite at a low temperature. Percentage of ash was calculated with reference to the air-dried drug[4].

$$\text{Total Ash value of the sample} = \frac{100\,(z - x)}{Y}\ \%$$

z = weight of the dish + ash (after complete incineration)
x = weight of the empty dish
y = weight of the drug taken

vi) Acid insoluble ash:

Boil the ash with 25ml of 2M hydrochloric acid for 5 minute, collect the insoluble matter in a Gooch crucible or on an ashless filter paper, wash with hot water, ignite, cool in a dessicator and weigh. Calculate the percentage of acid-insoluble ash with reference to the air dried drug[4].

vii) Water soluble ash:

To the crucible containing the total ash, add 25ml of water and boil for 5 minutes. Collect the insoluble matter in a sintered glass crucible or an ashless filter paper. Wash with hot water and ignite in a crucible for 15 minutes at a temperature not exceeding 450˚c. Subtract the weight of this residue in mg from the weight of total ash. Calculate the content of water soluble ash in mg/g of the air dried material[4].

viii) Determination of pH:

Determination of pH was carried out by using 1% solution of polyherbal formulation in distilled water and pH was determined using pH Systronic Digital pH Meter[3].

ix) Fluorescence analysis:

The fluorescence analysis was carried out by using 1 mg of polyherbal formulation on glass slide and treated with various reagents like $Fecl_3$, Conc.Hcl, HNO3, $K_2Cr_2O_7$, NaoH, $Agno_3$, Conc.H_2So_4,Conc. HNO_3, Br_2 water, 5% H_2O_2, CCl_4, Methanol, CHCOOH, Xylene, NH_3, I_2 for the presence of their fluorescence character under ultra-violet lamp[3].

x) Determination of viscosity, surface tension and density:

The determination of Density, surface tension and viscosity was carried out by using of 1% aqueous polyherbal formulation[3].

xi) Determination of Viscosity:

Ostwald viscometer is used to determine the viscosity of 1% polyherbal formulation. When a liquid flows by

gravity, the time required for the liquid to pass between two marks, through a vertical capillary tube is determined. The time of flow of the liquid under test is compared with the time required for a liquid of known viscosity (usually water). The viscosity of unknown liquid (η_1) can be determined using the following equation[5].

$$\eta_1 = \frac{P_1 t_1}{P_2 t_2} \times \eta_2$$

P_1 = density of the unknown liquid

t_1 = time of the flow of unknown liquid

P_2= density of the known liquid (density of water =0.9971g/ml)

t_2= time of the flow of known liquid

η_2 = viscosity of known liquid (water =0.8937cps)

xii) Determination of density:

Specific gravity bottle is used to determine the density of the 1% of polyherbal formulation The bottle is weighed, filled with the liquid whose specific gravity is to be found, and weighed again. The difference in weights is divided by the weight of an equal volume of water to give the specific gravity of the liquid[5].

Density of unknown liquid was calculated by using the following equation

$$\int_L = \frac{w_3 - w_1}{w_2 - w_1}$$

where $\int_L$ = density of unknown liquid

w_3 = weight of bottle + unknown liquid

w_1 = weight of empty bottle

w_2 = weight of bottle + water (density of water=0.9971g/ml)

xiii) Determination of Surface tension:

A stalagmometer is a device for investigating surface tension using the stalagmometric method. It is also called a stactometer or stalogometer. The device is a capillary glass tube whose middle section is widened. The volume of a drop can be predetermined by the design of the stalagmometer. The lower end of the tube is narrowed to force the fluid to fall out of the tube as a drop.[In an experiment, the drops of fluid flow slowly from the tube in a vertical direction. The drops hanging on the bottom of the tube start to fall when the volume of the drop reaches a maximum value that is dependent on the characteristics of the solution[5].

Surface tension of unknown liquid was calculated by using following equation:

$$\gamma_L = \frac{\eta_{H2O} \times d_{L1} \times \gamma_{H20}}{\eta_{L1} \times d_{H20}}$$

where :

η_{H2O} =Average number of drops of water

d_{L1} = Density of the unknown liquid

γ_{H20} = Surface tension of water

η_{L1} = Average number of drops of unknown liquid

d_{H20} = Density of water

V. Evaluation of Anti-depressant Activity:

a) Selection of Animals:

Swiss albino mice of either sex weighing (18-25g) and Albino wistar rats weighing (160-180g) were used for the study and were obtained from Drug testing laboratory, Bengaluru, India Ref.no. DCD/GCP/20/E.C/ADM/2015-2016 dated 05.03.2016.

b) Toxicity studies:

Acute toxicity studies were conducted as per internationally accepted protocol drawn under the OCED guidelines in swiss albino mice at a dose level of 2000mg/kg (Body Weight).

c) Forced Swim test:

Either sex of rats weighing (160-180g) were used. They are brought to the laboratory at least one day before the experiment and are housed separately in Makrolon cages with free access to food and water.

Rats were individually forced to swim inside a vertical Plexiglas cylinder (40cm; diameter: 18cm, containing 15cm of water maintained at 25˚c). Rats placed in cylinder for the first time were initially highly active, vigorously swimming in circles, trying to climb the wall or diving to the bottom. After 2-3 min activity begins to subside and to be interspersed with phases of immobility or floating of increasing length. After 5-6 min immobility reaches to a plateau where the rats remain immobile for approximately 80% of the time. The rats were removed and allowed to dry in heated enclosures (32 ˚c) before being returned to their home cages. They were again placed in cylinder 24h later and the total duration of immobility is measured during 6 min test. Floating behavior during 6 min period has been found to be reproducible in different groups of rats. An animal is judged to be immobile whenever it remains floating passively in the water in a slightly hunched but upright position, its nose just above the surface. The animals were divided into four groups of six each which includes control, standard and test group. Control group received 1% gum acacia, test group received polyherbal formulation of 200mg/kg, 400mg/kg (body weight) of animals, standard group received 5mg/kg i.p injected one hour prior to testing[6].

RESULTS:

Table-1 Extractive yield, nature, color and consistency of extracts.

Drug	Extractive yield (%)	Nature	Color
Ocimum sanctum	187.31%	Semi solid	Dark green
Centella asiatica	171.43%	Semi solid	Dark green
Curcuma longa	161,42%	Semi solid	Yellow
Withania somnifera	139.16%	Semi solid	Brownish yellow
Emblica officinalis	171.31%	Semi solid	Dark brown

Table-2 Phytochemical screening of polyherbal formulation:

Sl.no	Phytoconstituents	Name of test	Results
1	Alkaloids	Mayer's test Hager's test Wagner's test Dragendroff's test	– – + –
2	Glycosides	Legal's test	–
3	Phyto-sterols and terpenoids	Salkowaski test Liberman buchard test	+ +
4	Saponins	Foam test	+
5	Phenolic and Tannins	Ferric chloride test Lead acetate	+ –
6	Flavanoids	Shinoda test	–
7	Carbohydrates	Molischs test Benedicts test Fehlings test	+ – –
8	Proteins	Biuret test Ninhydrin test Millon's test	– – –
9	Fixed oils	Spot test	+
10	Test	Lignin Starch	+ +

" +" sign indicates **present**
" –" sign indicates **absent**

Table-3 Organoleptic properties of polyherbal formulation:

Color	Odor	Taste
Dark Brown	Slightly bitter	Characteristic

Table-4 Physio-chemical characteristic of polyherbal formulation

Sl.no	Parameter	%
1.	Water soluble extractive w/w	50%
2.	Alcohol soluble extractive w/w	20%
3.	Chloroform soluble extractive w/w	12%
4.	Pet ether soluble extractive value w/w	4%
5.	Ash content w/w	54.09%
6.	Acid insoluble w/w	16.5%
7.	Water soluble ash w/w	6.9%

Determination of PH:
Determination of pH was carried out by 1% of polyherbal formualtion solution in distilled water and pH is determined The pH is determined by Systronic Digital pH meter and was found to be 6.60

Table -5 Fluorescence Analysis of Polyherbal Formulation

Extract	Visible/day light	Ultra violet light
Extract+$Fecl_3$	Greenish Blue	Green colour
Extract+Conc. HCl	Black	Blue
Extract+10%HNO_3	Green	Green
Extract+10%$K_2cr_2o_7$	Greenish Yellow	Dark green
Extract+1m NaoH	Dark brown	Blackish green
Extract+$AgNo_3$	Slightly yellow	Light blue
Extract+Conc HNO_3	Greenish yellow	Dark green
Extract+ con H_2So_4	Reddish yellow	Slightly green
Extract+Br_2 Water	Red	Greenish blue
Extract + 5% H_2O_2	Yellow	Green
Extract+ CCl_4	Lightly yellow	Green
Extract+ Methanol	Yellow	Yellow
Extract+ CH_3CooH	Slightly yellow	Yellow
Extract+ Xylene	Yellow	Yellow
Extract+NH_3	Brown	Green
Extract+I_2	Blue	Light green

Table-6 Density, Viscosity and surface tension

Parameter	Values
Density (1%)	0.99
Viscosity (1%)	1.39cps
Surface Tension (1%)	40.88dynes/cm

Evaluation of Anti-depressant Activity:
Acute oral toxicity:
Polyherbal formulation did not produce any mortality even at the higher dose (2000mg/kg, p.o), on the basis of that the dose (200mg/kg) of formulation were selected for further pharmacological studies in animal models.

Forced Swim test:
Table-7 Effect of Polyherbal formulation on Forced swim test

Group No	Treatment on 1st day	Dose (kg-)	Immobility on 1st day
1	Control (1%gum Acacia)	2ml	226.66
2	Standard (Diazepam)	5mg	109.83
3	PF1	200mg	161.166
4	PF2	400mg	138.33

PF1= Polyherbal formulation 1
PF2 =Polyherbal Formulation

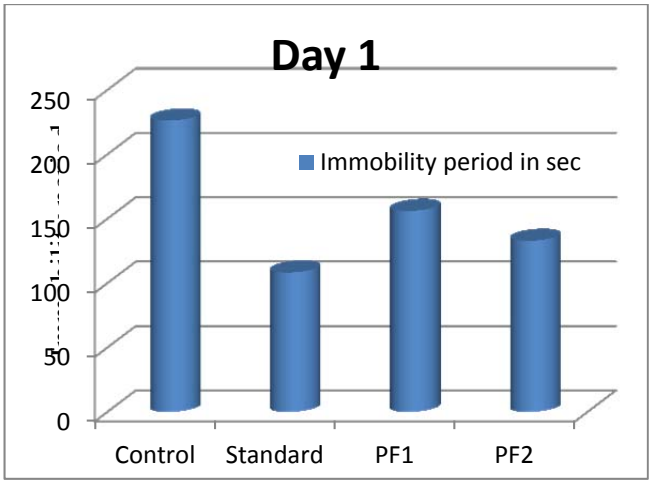
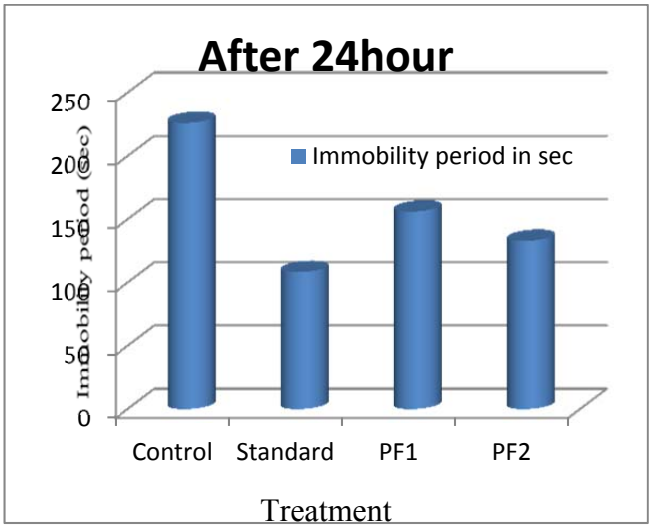


Table-8 Effect of Polyherbal formulation on Forced swim test

Group No	Treatment after 24h day	Dose (kg-)	Immobility after 24 h day
1	Control (1%gum Acacia)	2ml	226.33
2	Standard (Diazepam)	5mg	108.66
3	PF1	200mg	156.16
4	PF2	400mg	133

PF1= Polyherbal formulation 1
PF2 =Polyherbal Formulation



DISCUSSION AND CONCLUSION:

Mood disorder is one of the most common mental illness, with a life time risk of 10% in general population. Prevalence of depression alone in general population is estimated to be around 5% with suicide being one of the most common outcomes. Most of the drugs that are currently being used in the treatment of depression have adverse effects that affect the quality of life of the patient. This leads to patient's non-compliance to medication, which further complicates the problem[7].

The present study evaluated the anti-depressant activity of methanolic prepared polyherbal formulation in animal model of depression, forced swim test. This method is widely used for screening anti-depressant drugs. This test is quite sensitive and relatively specific to all major classes of anti-depressant like tricyclics, selective serotonin reuptake inhibitors (SSRIs), monoamine oxidase inhibitors (MAOIs) and atypical and atypical antidepressant.

The immobility reflects a state of despair in animals and is claimed to reproduce a condition similar to depression in humans. Animals after antidepressant treatment struggle more even in desperate situation and the spend less time with immobility[8].

Comparison of reduction in duration of immobility between 1st day and 24h showed that reduction immobility in different groups were more significant after 24h compared to 1st day.

The present study has shown that the polyherbal formulation at a dose of 400mg/kg significantly reduced the duration (time) of immobility of animals as compared to the control in forced swim test[9].

ACKNOWLEDGEMENT:

My heartfelt thanks to AAGCPA members for funding my research project and utilization for different instruments and equipment in Department of Pharmacognosy and Department of Pharmacology, Government college of Pharmacy Bengaluru-27

REFERENCE:

1. Anindita DE and Singh M. *Acorucs calamus* linn. Rhizome extract for antidepressant activity in mice model. Advance Research in Pharmaceuticals and Biologicals.2013;vol 3(4):520-25.
2. Dhingra D and Sharma A. A review on antidepressant plants. Natural products Radiance.2005:144-152.
3. Madhav SNV, Upadhyaya K, Bisht A. Phytochemical screening and standardization of polyherbal formulation for dyslipidemia. International Journal of Pharmacy and Pharmaceutical Sciences. 2011;vol3(3):235-38.
4. Mukherjee PK. Quality Control of Herbal Drugs. Business Horizons Pharmaceutical Kolkata.5th ed.2012:186-90.
5. Subrahmanyam CVS. Text Book of Physical Pharmaceutics. Vallabh Prakashan Publication.13th ed. Delhi:133,215,259.
6. Gerhard Vogel H, Wolfgang, Vogel H. Drug discovery and evaluation of pharmacological assays. 2nd ed. Germany: 1997;434-35.
7. Pemminati S, Gopalakrishna HN, Shenoy AK, Sahu SS,Mishra S, et al. Antidepressant activity of aqueous extract of fruit of *Emblica officinalis* in mice. International Journal of Applied Biology and Pharmaceutical Technology.2010;vol1(2):449-54.
8. Bharathi P, Seshayamma V, Jagannadharao GH. Evaluation of antidepressant activity of aqueous extract of *Withania somnifera* roots in albino mice. IOSR Journal of Pharmacy and Biological Sciences.2015;vol10(1):27-9.
9. Baht NP, Parashivamurthy BM. Evaluation of antidepressant like activity of aqueous extract of fruit of *Emblica officinalis* in albino mice. International Journal of Recent Trends in Science and Technology.2013;vol9(2):290-94.