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Evaluation of anxiolytic effect of a polyherbal formulation in mice

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ABSTRACT

In present modern life style anxiety 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 anxiolytic effect of polyherbal formulation and synergism between compounds present in formulation. Formulation consists of hydrolacoholic extract of Centella asiatica, Withania somnifera and Ocimum sanctum all of which are classified in Ayurveda as rasayanas which are reported to promote physical and mental health. Individual extracts and formulation were screened for phytochemical investigation and anxiolytic action in mice. Phytochemical tests were carried out by using standard reported methods which revealed the presence of desired phytoconstituents for anxiolytic action. In elevated plus maze model and Light/dark exploration model, formulation has revealed the significant anxiolytic activity at 200 mg/kg. Formulation has shown potent activity than individual plant extracts. Thus present study revealed that formulation showed anxiolytic activity and this may be due to synergism between Centella asiatica, Withania somnifera and Ocimum sanctum.

Key words: Anxiolytic, polyherbal, *Centella asiatica*, *Withania somnifera* and *Ocimum sanctum*

INTRODUCTION

Anxiety is a human emotion that serves an adaptive function from a psychobiological perspective. However, in the psychiatric setting, feelings of fear or dread that are unfocused or out scale with perceived threat often require treatment .Typically, the psychic awareness of anxiety is accompanied by enhanced vigilance, motor tension, and autonomic hyperactivity. Anxiety is often secondary to organic disease states—acute myocardial infarction, angina pectoris, gastrointestinal ulcers, etc—which themselves require specific therapy. [1]

Till date, the effective drugs without side effects for the anxiety are very limited so the need for newer, better-tolerated and more efficacious treatments is remaining high. The most widely prescribed medications for anxiety disorders are the benzodiazepines. However, the clinical uses of benzodiazepines are limited by their side effects

such as psychomotor impairment, potentiating of other central depressant drugs and dependence liability. Therefore, herbal therapies should be considered as alternative/ complementary medicines. Recently, the search for novel pharmacotherapy from medicinal plants for psychiatric illnesses has progressed significantly. This has been reflected in the large number of herbal medicines whose psychotherapeutic potential has been assessed in a variety of animal models. [2]

Herbal drugs in the recent years have gained great importance because of their efficacy and easy availability. *Rasayana* drugs in the Ayurvedic literature were used in various ailments in the folklore and indigenous system of medicines since times immemorial. These drugs were also employed to prevent stress, ageing, increase longevity, anxiety and offer resistance to diseases by augmenting the immune system. [3]

Drug discovery and development need not always be confined to new molecular entities. Rationally designed, carefully standardized, synergistic traditional herbal formulations and botanical drug products with robust scientific evidence can also be alternatives. A reverse pharmacology approach, inspired by traditional medicine and Ayurveda, can offer a smart strategy for new drug candidates to facilitate discovery process and also for the development of rational synergistic botanical formulation**.** [4]

Thus, by considering all these facts the present study was such an attempt to develop polyherbal formulation for anxiety management with minimum ingredients to ensure standardization.

MATERIALS AND METHODS

Plant material and extraction of drug

The plants of *C. asiatica, O. sanctum, W. somnifera* were collected from Pune,Maharshtra,India and authentication of the plants was done by Dr.Rajesh Dabur,Regional Research Institute(AY.)Pune. Hydroalcoholic extracts of whole herb of *C. asiatica*,leaves of *O. sanctum* and roots of *W. somnifera* were prepared separately by hot percolation method through soxhlet apparatus. Thereafter extracts were dried by using rotary vaccume evaporator. The amount of extract were weighed and stored in amber colored airtight bottles.

Preliminary phytochemical screening of crude extracts

The hydroalcoholic extracts of three plants material were subjected to qualitative tests in order to identify class of compound by using preliminary phytochemical test. [5]

TLC profile of plant extracts:

TLC studies were performed for presence of principal constituents by using reported methods. The hydroalcoholic extracts of *C. asiatica, O. sanctum, W. somnifera* were dissolved in 70% ethanol. The reported solvent systems used for establishing the profiles. Solvent system used for *C .asiatica* was n-Butanol: Ethyl acetate: Water (4:1:5) [6**]** for *W. somnifera,* Toluene: Ethyl acetate: Formic acid (5:5:1) [7] and for *O. sanctum* Petroleum ether: Ethyl acetate: Formic acid. (93:7:0.7)**.** [8] The Rf value of each resoluted compound was recorded.

Composition and preparation of tablet formulation:

Tablet formulation (400mg) was prepared which contain excepients viz mannitol (84mg), Sodium starch glycolate (16mg), Magnesium stearate(8mg), Talc(20mg), Aerosil(12mg), Povidone(20mg) and extracts of whole plant of *C. asiatica* (80 mg), roots of *W. somnifera* (80 mg), leaves of *O. sanctum* (80 mg). *C. asiatica*, *W. somnifera*, *O. sanctum* extracts were passes through 40 mesh sieve. Mannitol and half quantity of sodium starch glycolate were passed through 40 mesh sieve and added to active drugs. Povidone was also dissolved in isopropyl alcohol. These were added to active drugs. Proper mixing of all above formed weight mass .This weight mass was passed through 10 mesh sieve to form granules. Granules were air dried at room temperature. After drying granules were passed through 20 mesh sieve. Talc, Aerosil was added to granules by passing through 60 mesh sieve and remaining half quantity of disintegrant was added. Magnesium stearate was passed through 60 mesh sieve and added at last. Compression of granules was done to form tablets by using tablet punching machine**.** [9]

Selection and maintenance of animals:

Swiss albino mice of either sex weighing 18-25 gm were obtained from National Toxicology Center, Pune, India. Mice were maintained at standard laboratory conditions.

Toxicity study:
Acute toxicity studies were conducted as per the internationally accepted protocol drawn under the OECD guidelines in swiss albino mice at a dose level of tablet formulation 1000mg/kg.

Elevated plus maze model:
The animals were divided into eight groups consisting of six animals in each. Group I received vehicle (1% SCMC).Group II was positive control and received standard Gerifort (43 mg/kg p.o.). Group III, IV and V received extracts of individual drugs (*C. asiatica*, *W. somnifera* and *O. sanctum* respectively) at dose 80mg/kg p.o. and Group VI, VII, VIII received tablet formulation at dose 50,100,200 mg/kg p.o. respectively. All the groups were treated with their respective treatment for 1 day.

After 30 min of treatment mice were placed individually in the centre of the maze, facing the closed arm. The time spent in both the open and closed arms was recorded for 5 min. The numbers of entries into the open and closed arm were also counted during the test. An entry was defined as having all four paws within the arm**.** [10- 11]

Light/dark exploration test:
The apparatus consisted of two acrylic boxes. Two distinct chambers, a black chamber (20 x 30 x 30 cm) painted black and other open chamber made up transparent acrylic (30 x 30 x 30cm). The two chambers are connected through a small open doorway (8x 8 cm) situated on the floor level at the centre of the partition. One box was made dark by covering its top with plywood and a 10W lamp illuminated the other box. The light source was placed 25 cm above the open box. The mice were placed individually in the center of the lit box and observed for the next 5 min for the time spent in lit and dark boxes. Each mouse was placed individually in the light compartment and observed or the next 5 minutes for the numbers of the crossing between two compartment and time spend in the light and dark compartment.[2]

Statistical analysis:
The results are expressed as Mean ± S.E.M. and subjected to one way Analysis of Variance (ANOVA) followed by Tukey- Kramar multiple comparisons test using INTA software.

RESULTS

Preliminary phytochemical screening and TLC profile of plant extracts
Preliminary phytochemical screening and TLC profile of plant extracts showed presence of desired phytoconstituents in each extract and results are shown in the table 1 and 2.

Acute oral toxicity:
Tablet formulation did not produce any mortality even at the highest dose (1000 mg/kg, p.o.), on the basis of that three doses (50,100 and 200mg/kg) of formulation were selected for further pharmacological studies in animal models.

Elevated plus maze test:
In this model number of entries and duration of stay in open arm and closed arm were measured results obtained are in the table No: 3.Gerifort 43mg/kg ($p<0.001$) and tablet formulation 200mg/kg ($p<0.01$) showed significantly increased the number of entries and duration of stay in open arm. While other groups does not showed significant results. All treated groups were compared with control.

Light/dark exploration test:
In this model the time spent and number of entries in light compartment were measured results obtained are in the table 4. Gerifort 43mg/kg ($p<0.001$) and tablet formulation 200mg/kg ($p<0.01$) showed significantly increased the time spent and numbers of entries in light compartment while other groups does not showed significant results. All treated groups were compared with control.

DISCUSSION

Anxiety is a negative emotion that occurs in response to perceived threats can come from internal or external factors and can be real or imaginary. The incident of the anxiety in the community is very high and associated with many

diseases and morbidity. Ethanopharmacological knowledge about plants under study would allow us to evaluate central nervous system activity [12]. The etiology of most anxiety disorders are not fully understood, but various studies has shown the involvement of GABAergic, serotonergic neurotransmission in etiology, expression and treatment of anxiety. The adrenergic and dopaminergic systems have also been shown toplay a role in anxiety [13]. So the present study was aimed was an attempt to develop synergistic polyherbal formulation for anxiety management from plants used in the Indian System of Medicine(ISM) and which are scientifically proved as potential anxiolytic agent.

Qualitative phytochemical tests of hydroalcoholic extract of *C.asiatica, W.somnifera, O.sanctum* revealed the presence of carbohydrates, glycoside, flavonoid, terpenes, steroids, saponin, alkaloids and phenolic compound. In TLC study extract of *C.asiatica* showed presence of triterpen saponine, *W.somnifera* showed presence of withanolides and *O.sanctum* showed presence of triterpenoid and these compounds are reported for potent anxiolytic action.

The elevated plus-maze (EPM) is considered as one of the valid ethological animal models of anxiety since it employs natural stimuli (fear of a novel, brightly lit open space and fear of balance of a relatively narrow and raised platform) capable of inducing anxiety in humans. [14] In Elevated plus maze normally animals prefer to spend much of their allotted time in the closed arm. This preference appears to reflect an aversion towards the open arms that is generated by fear of open spaces. Drugs that increase the open arm exploration are considered as anxiolytic and the reverse holds true for anxiogenics.[10]

The anxiolytic action was also observed in case Light/dark exploration test. This test is an ethological based approach avoidance conflict test and it is sensitive to drugs that affect the anxiety. [12] Mice tend to explore a novel environment, but to retreat from the aversive properties of a brightly-lit open field. In a two chambered system where the animals can freely move between a brightly-lit open field and a dark corner, they show more increasing time spending action in light chamber due to anxiolytic agents. [1]

In both models Gerifort 43mg/kg and tablet Formulations 200mg/kg showed significant anxiolytic property while individual plants did not show significant results this may be due synergistic effect of triterpenoids present in the *O.sanctum*, asiaticoside and other terpens present in C.*asiatica* [15] glycowithanolides present in *W. somnifera* [16] Thus present study reveals that Formulation has potent anxiolytic activity.

Table1: Phytochemical evaluation of extracts of *C. asiatica, W. somnifera, O.sanctum*

Test	*C. asiatica*	*W. somnifera*	*O. sanctum*
Carbohydrate	+	+	+
Protein/amino acid	+	+	+
Glycoside	+	+	+
Flavonoids	+	-	+
Alkaloids	-	+	-
Terpenes	+	+	+
Steroids	+	+	+
Saponin	+	+	-
Phenolics /Tannins	+	-	+

(+) indicates presence
(-) indicates absence

Table 2. TLC of extracts of *C. asiatica, W. somnifera, O. sanctum*

Sr. no	Drug	Solvent system used	Colour observed	R_f	Compound may be
1.	*C. asiatica*	Chloroform-Glacial acetic acid-Methanol-Water (60:32: 0.8)	Bluish violet spots	0.35	Asiaticoside
2	*W. somnifera*	Toluene: Ethyl acetate: formic acid (5:5:1)	Bluish violet spots	0.5	Withanolide A
3.	*O. sanctum*	Petroleum ether-ethyl acetate-Formic acid (93:7:0.7)	Bluish violet spots	0.23	Ursolic acid

Table: 3. Results of elevated plus maize model.

Sr. No.	Group	(Dose/ day)	Time spent in open arm(s) Mean ± S.E.M.	Time spend in closed arm(s) Mean± S.E.M.	No. of entries in open arm(s) Mean ± S.E.M.	No of entries in closed arm(s) Mean ± S.E.M.
1	Control	1% SCMC	75.33 ± 5.69	205.50 ± 5.11	5.33 ± 0.66	14.66 ± 0.66
3	Gerifort	43mg/kg(p.o.)	119.83 ±5.73***	160.33±3.26***	18.5 ±1.83***	6.66±0.76***
4	*C. asiatica* extract	80mg/kg(p.o.)	82.33 ± 7.37	200 ± 6.66	7 ± 0.63	12.83 ± 0.94
5	*O .sanctum* extract	80mg/kg(p.o.)	80.16 ± 6.22	204 ± 3.62	6 ± 0.93	13.66 ± 1.20
6	*W. somnifera* extract	80mg/kg(p.o.)	78.16 ± 8.78	197.83 ± 7.13	5.83 ± 0.90	14 ± 1.23
7	Formulation 50	50mg/kg(p.o.)	85 ± 7.78	195.50 ± 10.32	8.83 ± 1.24	12.50 ± 1.47
8	Formulation 100	100mg/kg(p.o.)	101.67 ± 5.23	177.17 ± 5.26	10.16 ± 1.53	9.16 ± 1.10
9	Formulation 200	200mg/kg(p.o.)	112.17 ± 6.27**	168.50 ± 6.59**	13 ± 1.34**	7.66 ± 1.52**

n= 6

*Values are expressed as Mean ±S.E.M. * = P<0.05, ** = p<0.01 and *** = p<0.001 Test drug treated groups were compared with Control Group. (Statistically analysed by one way ANOVA followed by Tuky- Kramar multiple comparisons test.)*

Table 4. Results of Light/dark exploration test

Sr. No.	Group	(Dose/ day)	Time spent in light area(sec) Mean ± S.E.M.	No. of entries in open arm(s) Mean ± S.E.M.
1	Control	1% SCMC	73.33 ± 4.67	7.35 ± 0.67
3	Gerifort	43mg/kg(p.o.)	140.83 ±6.73***	17.5 ±1.60***
4	*C. asiatica* extract	80mg/kg(p.o.)	82.33 ± 6.37	6 ± 0.63
5	*O .sanctum* extract	80mg/kg(p.o.)	78.16 ± 6.22	7 ± 0.83
6	*W. somnifera* extract	80mg/kg(p.o.)	79.16 ± 8.67	6.83 ± 0.70
7	Formulation 50	50mg/kg(p.o.)	87 ± 6.79	8.50 ± 1.10
8	Formulation 100	100mg/kg(p.o.)	98.67 ± 5.24	11.12 ± 1.43
9	Formulation 200	200mg/kg(p.o.)	110.17 ± 6.27**	14 ± 1.24**

n= 6

*Values are expressed as Mean ±S.E.M. * = P<0.05, ** = p<0.01 and *** = p<0.001 Test drug treated groups were compared with Control Group. (Statistically analysed by one way ANOVA followed by Tuky- Kramar multiple comparisons test.)*

CONCLUSION

Study shown that formulation was superior than individual plants i.e. *C. asiatcia W. somnifera* and *O. sanctum* this clearly suggest that synergism take place and hence formulation is advised in anxiety management. Since this formulation contain minimum ingredients it will be easy to ensure standardization of formulation. Detailed biological investigation and mechanism of actions of synergistic effect of formulation and clinical trails on human being need to be carry out in order to explain therapeutic use of formulation.

REFERENCES

[1] Jyothi CH et al, *Journal of Pharmacy Research*, **2012,** 5(3), 1624-1627.
[2] Jaspreet Nain et al, *Journal of Pharmacy Research*, **2011**, 4(12), 4485-4487.
[3] B.Singh, B.Chandana, N.Sharma, S. Singh, A. Khajuriaa, D.Gupta, *Phytomedicine*, **2005,** 12,468–481.
[4] B.Patwardhan, R.A.Mashelkar, *Drug Discovery Today*, **2009**, 14(15-16), 804-11.
[5] K.R. Khandelwal, Practical Pharmacognosy Techniques and Experiments, Tenth edition, Nirali Prakashan, Pune, **2003,** 149-158.
[6] V.Rajpal. Standardization of botanicals. First edition Vol II, Estern publisher, Mumbai, **2004**, 229-238.
[7] V.Sharma, A.P. Gupta, Bhandari, R.C.Gupta, B.A.Sing, Chromatographia, **2007, 66,**801-804.
[8] E.Stahl, Thin layer chromatography, A Laboratory handbook, First edition, Springer International, New Delhi, **2005**,244.
[9] H.C. Ansel, Pharmaceutical Dosage forms and Drug Delivery Systems, Eight edition, Wolter Kluwer Pvt Ltd, New Delhi, **2005**,193-203.
[10] A.V.Yadav,L.A. Kawale,V.S. Nade, *Indian J. of Pharmacol*. **2008**, 40 (1), 32-36.
[11] I. Soman, S.A. Mengi, S.B. Kasture, *Pharmacology,Biochemistry and Behavior*,**2004**,79,11-16.
[12] Thippeswamy et al, *Indian J. of Pharmacol*, **2011,** 43(1), 50-55.
[13] Habibur R, Muralidharan1 P, Sivaraman1 D, Kartika B, Dipankar S, *Der Pharmacia Sinica***, 2010**, 1 (3): 86-94.
[14] Eric W, Patrick A, Wonder KM, Der Pharmacia Sinica **2011**, 2 (2): 54-67.

[15] P.Wijeweeraa, J.T.Arnasona,D. Koszyckib,Z. Meralib, *Phytomedicine*,**2006**, 13,668–676.
[16] M.Hussain, A.Abdul-Hamid,S. Mohamad,N. Saari, M.Ismail,M.H. Bejo, *Food Chemistry*,**2007,** 100,535–541.