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# Development of Mono Ingredient Herbal Neuroleptic Tablet for Better Psychiatric Therapy

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**Conventional synthetic neuroleptic drugs used for the treatment of chronic cases of psychosis are associated with extra pyramidal side effects. Herbal neuroleptic drugs might prove to be devoid of these side effects. Monoherbal and polyherbal neuroleptic tablets of various combinations were prepared using *Acorus calamus* (I), *Glycyrrhiza glabra* (II) and *Withania somnifera* (III). Wet granulation technique was employed for tablet formulation (100 mg) and the tablets were film coated with hydroxy propyl methyl cellulose. The qualitative and quantitative estimation of various active constituents was done by high performance thin layer chromatography where elution media such as toluene:ethyl acetate (93:7) for I, methanol:strong ammonia (200:3) for II and ethyl acetate:ethanol:water:ammonia (65:25:9:1) for III were used. The amount of β-asarone, glycyrrhizic acid and withaferin-A present in 100 mg of mono ingredient herbal tablets were found to be 2.81%, 2.95% and 0.69%, respectively. Tranquilizing effect, anxiolytic efficacy and catatonic response were measured in wistar rats. An optimum therapeutic efficacy of mono herbal formulations were confirmed by sleeping tranquilizing effects ($P<0.001$) using rotarod method and anxiolytic effects by thiopental sleeping time on Wistar rat model. No drug-induced catatonia was observed with the developed tablet formulations in rat model. The monoherbal tablet dosage form thus prepared may be a better alternative over synthetic drugs and polyherbal formulations during chronic treatment of psychosis and thereby expected to be a cost effective, safe and natural product for better psychiatric therapy.**

Synthetic antipsychotic drugs are associated with self induced extra pyramidal side effects (EPS)[1,2]. The literatures support that newer dosage form of these synthetic drugs might provide better efficacy[3,4] and atypical neuroleptic drugs may be devoid of this problem[5]. Herbal drugs used for the same purpose are believed to be safe[6] with little or no effects on extrapyramidal systems. An investigation is made on three plant species, which are occurring in nature and traditionally used as neuroleptic agents. *Acorus calamus* (Araceae) rhizome contains β-asarone, which is indicated in epilepsy, delirium, amnesia, convulsion, depression and other mental disorders and is used as a sedative, nerve tonic and tranquilizer[7,8]. *Withania somnifera* (Solanaceae) has a sedative and hypnotic effect due to withaferin-A and is also indicated in hypertension, respiratory stimulation, bradycardia, rheumatism and gout. The alkaloids present produce a taming and a mild depression effect on CNS[9]. *Glycyrrhiza glabra* (Leguminaceae) contains an active drug constituent called glycyrrhizic acid, which is useful in intellect promoting, tranquilizing influence on CNS, expectorant, aphrodisiac and urecosis[10].

In the present investigation, the qualitative and quantitative estimations of β-asarone, withaferin-A and glycyrrhizic acid in prepared herbal tablets were determined by high performance thin layer chromatography (HPTLC) and verified the same from their individual reference markers. It was also compared for their therapeutic efficacy and drug-induced EPS over marketed formulation and synthetic drug

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in animal models. The potency of individual herbal drugs was compared and its efficacy was determined over polyherbal systems. As the use of these plant species in psychiatric treatment are in traditional practices, the present study was undertaken to evaluate the psychopharmacological potential of these drugs in tablet form and to gather the information of their beneficial efficacies over synthetic drug, especially in mono ingredient system.

## MATERIALS AND METHODS

The drugs, *Acorus calamus*, *Withania somnifera*, and *Glycyrrhiza glabra* are commonly available and the dried roots and rhizomes were collected from Coimbatore, Tamilnadu. Methanol was obtained from Qualigens Fine Chemicals, Mumbai. Ethyl acetate, toluene, n-hexane and acetone were obtained from Fischer Inorganics and Aromatics Ltd., Chennai. Strong ammonia and chloroform were obtained from Ranbaxy Laboratories, New Delhi. HPTLC pre coated plates were obtained from E. Merck (India) Ltd., Mumbai. Other excipients like starch, lactose, vanillin, PEG 400, magnesium stearate, erythrosin and spraying reagents like anisaldehyde, Dragendroff's reagent were obtained from S. D. Fine Chemicals Ltd., Boisar. Talc was obtained from Ajay Enterprises, Chennai.

### Extraction:

The collected roots and rhizomes of the plants were washed under running tap water, scrapped into small pieces and dried at an ambient temperature of 50°. The dried material was comminuted into coarse powder using a suitable mill and fine powders were separated by passing through BSS sieve number 60. A known weight of powdered drug was taken in a soxhlet apparatus and extracted with methanol (99% v/v). The extracts were dried under vacuum for individual plant drugs. The yields of alcoholic extracts were found to be 22.5%, 8.18% and 18.2 % for *Acorus calamus*, *Withania somnifera* and *Glycyrrhiza glabra*, respectively.

### Tablet compression and film coating:

The tablets were made by wet granulation technique using 11/32 punch in a Rimek mini press I tablet machine (Karnavathi Engineering, Ahmedabad.) with a compressional load of 4.75 tons. Various batches of tablet formulations (100 mg) were prepared by using individual drug extracts and their combinations (Table 1). Other excipients used were, drug:lactose (1:0.5), magnesium stearate 2% and sufficient starch paste. Simple film coating was done with HPMC, PEG 400 by spraying technique of the moving bed of compressed tablet using Kalweka coating pan. The physical assessments of the tablets were performed by determining various evaluation parameters which include hardness, friability and disintegration using standard procedures[11].

### Qualitative and quantitative determination of extracts by HPTLC:

The standardized extracts containing β-asarone, withaferin-A and glycyrrhizic acid were obtained from the sample bank of Kancor Flavours and Extracts Ltd., Angamali, Kerala. The standards were prepared by dissolv-

TABLE 1: BATCH FORMULATION AND PHYSICAL PROPERTIES OF TABLETS

| Batch code | Batch combination A:B:C (100 mg) | Friability (%w/w) | | Hardness (Kg/cm²) Starch (%) | | | | Disintegration Time (min) starch (%) | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | UC | FC | 6 | | 10 | | 6 | | 10 | |
| | | | | UC | FC | UC | FC | UC | FC | UC | FC |
| Batch–I | 1:1:1 | 0.892 | 0.003 | 4.0 | 4.8 | 5.1 | 5.7 | 4 | 8 | 5 | 10 |
| Batch-II | 1:0:0 | 0.518 | 0.101 | 4.8 | 5.6 | 5.3 | 6.0 | 5 | 8 | 6 | 11 |
| Batch–III | 0:1:0 | 0.728 | 0.015 | 4.6 | 5.4 | 5.6 | 6.4 | 4 | 9 | 6 | 10 |
| Batch- IV | 0:0:1 | 0.623 | 0.005 | 3.8 | 4.3 | 5.0 | 5.9 | 4 | 7 | 5 | 9 |
| Batch-V | 1:1:0 | 0.727 | 0.89 | 3.9 | 4.1 | 5.1 | 5.7 | 4 | 9 | 6 | 11 |
| Batch-VI | 0:1:1 | 0.675 | 0.909 | 4.2 | 4.6 | 5.5 | 5.9 | 5 | 8 | 5 | 10 |
| Batch-VII | 1:0:1 | 0.984 | 0.009 | 4.4 | 4.7 | 5.2 | 5.4 | 4 | 7 | 6 | 12 |

UC, FC represents uncoated and film coated tablets. A, B, C refers to *A. calamus*, *G. glabra* and *W. somnifera*, respectively.

ing the extracts in suitable solvents. *Acorus calamus* extract (100 mg) was dissolved in 50 ml of toluene. *W. somnifera* extract (100 mg) was dissolved in 10 ml of methanol and *G. glabra* extract (100 mg) in 10 ml of 50% v/v ethanol. All the standards were scanned and the corresponding λ max was found to be 215 nm, 248 nm and 298 nm, respectively. The crude extracts (100 mg) of roots and rhizomes of the plants and the tablet formulations (100 mg) were dissolved in the similar manner as that of the standards.

**Conditions of chromatography:**

HPTLC pre coated silica gel 60 G (Merck) test plates were used. Spotting volumes taken were 2, 4, 6, 8 and 10 µl for standard and 5 µl for samples of *A. calamus* and 10 µl each for both standard and samples of *W. somnifera and G. glabra*. Twin trough glass chambers 12x12 cm (Camag, Switzerland), were used as development chamber. Mobile phases used were, toluene: ethyl acetate (93:7) for *Acorus calamus*, methanol: strong ammonia (200:3) for *W. somnifera and* ethyl acetate: ethanol: water: ammonia (65:25:9:1) for *G. glabra*. Densitometry UV scanning (Camag scanner III) was done in absorbance reflection mode.

**Tranquilizing efficacy by rotarod method:**

The therapeutic efficacy of the tablets was verified on rat model using rotarod method to determine the tranquilizing effects[12,13]. Adult Wistar rats (100-180 g) were used after getting proper approval from the Institutional Animal Ethics Committee (IAEC, Approval no. 26/29, dated 16.12.2000). Thirty five Wistar rats were weighed individually in a physical balance and they were divided into seven groups, each containing five animals. One group received only 0.5% carboxy methyl cellulose (CMC) and the second group received 3 mg/kg of chlorpromazine. The other groups received 150 mg/kg of prepared formulations, out of which one group received 150 mg/kg of marketed formulation orally. Rats were placed on an aluminium rod of 2 cm in diameter, rotating at a speed of 20 rpm. Circular section divides the linear space of the rod into four lengths, so that four rats can be tested together. The controlled and treated rats were placed on the rod at intervals and the time of the fall from the rod was noted. The test was terminated at 300 s.

**Anxiolytic efficacy by thiopental sleeping time:**

The anxiolytic activity was determined by comparing the sleeping time of the formulations with thiopental sodium as a reference standard drug[14-16]. Thirty Wistar rats (100-180 g) were weighed individually in a physical balance and they were divided into six groups, each containing five animals. Herbal formulations were administered orally (150 mg/kg) to the four groups and one group received marketed formulation (150 mg/kg) orally. The last group received thiopental sodium (5 mg/kg) intraperitoneally. Effects of various extracts and thiopental sodium on sleeping time were determined. Onset and duration of actions of thiopental and herbal formulations were calculated. Potentiation of hypnotic effect was found by determining the effects of various extracts on thiopental sodium sleeping time. For this, thiopental sodium was administered thirty minutes after the administration of herbal formulations to the same group of rats and there after onset and duration of actions were measured.

**Study of drug-induced catatonic response:**

The drug-induced EPS study[17] was carried out by determining the catatonic response in Wistar rats (100-200 g). The rats were divided into seven groups consisting of five animals in each group. One group received 0.5% CMC, the other group received chlorpromazine (5 mg/kg) and the remaining groups received 150 mg/kg of herbal tablets. After administration of drugs to animals, severity of catatonic response was observed. Stage I: rat moves normally when placed on the table, score = 0. Stage II: rat moves only when touched or pushed, score = 0.5. Stage III: rat placed on the table with alternating front paws on a 3 cm high block fails to correct the posture in 10 seconds, score = 0x5 for each paw with a total score of 1 for this stage. Stage IV: rat fails to remove its front paws when placed alternatively on a 9 cm block, score = 1 for each paw with a total score of 2 for this stage. The severity of catatonia at 1, 2, 4, 8, 16 and 24 h after the administration was observed.

## RESULTS

The compressed tablets after film coating with HPMC were found very elegant and had a commercial look. The average weight of tablets of different formulations was found to be 0.28 g. The average thickness of each tablet was found to be 0.39±0.05 cm. The friability value of different formulations was within 1%. With 6% starch, the hardness of uncoated and film coated tablets were found to be in the range of 3.5-5 kg/cm² and 4-6 kg/cm², respectively. The disintegration time for uncoated and film coated tablets was found to be 4-5 min and 7-10 min as per USP, respectively.

The prepared extracts were subjected to qualitative estimation using pre coated HPTLC plates. Fingerprints were taken for all the extracts and compared with the chromatogram of standardized extracts. Both the fingerprints

TABLE 2: QUANTITATIVE DETERMINATION OF ACTIVE CONSTITUENTS OF HERBAL DRUGS IN CONCERNED FORMULATIONS BY HPTLC

| Drug | | AEP | Standard | | | Sample | | AACP | AACP | % |
|---|---|---|---|---|---|---|---|---|---|---|
| Active constituent | IC | (mg) | C (mg/ml) | AA (μl) | AUC | AA (μl) | AUC | sample (μg) | Tablet (mg) | AACP Tablet |
| β-Asarone | A | 100 | 0.6 | 2 | 8033.8 | 5 | 12573.1 | 2.810 | 2.810 | 2.81 |
| | | | | 4 | 12333.8 | | | | | |
| | A/W/G | 33.33 | | 6 | 15388.8 | 5 | 5122.3 | 0.850 | 0.850 | 2.55 |
| | | | | 8 | 17681.3 | | | | | |
| | | | | 10 | 19560.1 | | | | | |
| Withaferin-A | W | 100 | 0.2 | 10 | 26709.7 | 10 | 9182.3 | 0.687 | 0.687 | 0.69 |
| | A/W/G | 33.33 | | | | 10 | 2808.7 | 0.210 | 0.210 | 0.63 |
| Glycyrr | G | 100 | 0.6 | 10 | 16538.0 | 10 | 8144.8 | 2.95 | 2.950 | 2.95 |
| hizic acid | A/W/G | 33.33 | | | | 10 | 3404.2 | 1.24 | 1.240 | 3.72 |

A, G, W indicates *A. calamus, G. glabra* and *W. somnifera*. AUC stands for Area under the curve and C refers to concentration. IC refers to individual and combination of extracts present in one tablet, AEP represents amount of extract present, AA refers to amount applied and AACP refers to amount of active constituent present.

were matched and the authenticities of the drugs were confirmed. The HPTLC absorption spectra of the three drugs were determined in Camag, Scanner III machine and found that λ max for withaferin-A, Glycyrrhizic acid and β-asarone was found to be 215 nm, 248 nm and 298 nm, respectively. Table 2 shows various active constituents estimated by multi level and single level calibration. In case of *A. calamus*, by multi level calibration with active constituents, through continuous reflux of extract with selected solvent, it was found that the amount of β- asarone present in 100 mg and 33.3 mg tablets were found to be 2.81 mg and 0.85 mg, respectively. *G. glabra* and *W. somnifera* were standardized using single level calibration method and was found that 100 mg and 33.3 mg tablets of *W. somnifera* contain 0.687 mg and 0.210 mg of *withaferin-A* and 2.95 mg and 1.24 mg of glycyrrhizic acid were present in 100 mg and 33.3 mg of *G. glabra* tablets.

The tranquilizing efficacies of herbal tablet formulations were determined by rotarod method. The results show that the falling time was significantly less when compared to the control with higher't' values ($p<0.001$) determined from Dunnett's 't' test[18]. This indicates that the tablet formulations containing *A. calamus, W. somnifera* and *G. glabra* were having sufficient neuroleptic effects. The sedative and hypnotic activity (righting reflex) was determined by comparing the sleeping time of herbal formulations with thiopental sodium as a reference standard. The results indicated that the extract at the dose level of 150 mg/kg body weight showed significant hypnotic activity except *G. glabra*. In case of drug induced catatonia, the results indicated that there was no catatonic response (score=0) with the formulated tablets during a period of 24 h study, when compared with a positive control of chlorpromazine (maximum score=3.5). This means no major drug-induced EPS were present in the formulations.

## DISCUSSION

The results obtained from this investigation revealed that the dried extracts of the three plant species namely *A. calamus, W. somnifera and G. glabra* are suitable for tablet formulations in various combinations and gave a stable, non hygroscopic and elegant film coated tablet intended for oral administration. The individual active constituents were determined by suitable HPTLC method and optimum drug concentrations were found in each tablet formulations. In order to find out the therapeutic efficacy, the individual tablet formulations were crushed into powders, suspended in carboxy methyl cellulose and orally administered to rats. The tranquilizing efficacy was determined by rotarod method and found a substantial decrease in falling time. The anxiolytic property by thiopental induced sleeping time

TABLE 3: EVALUATION FOR NEUROLEPTIC EFFICACY AND DRUG-INDUCED CATATONIA OF DIFFERENT FORMULATIONS

| Group | Treatment | Tranquilizing efficiency | Assessment of anxiolytic property | | Degree of catatonia |
|---|---|---|---|---|---|
| | | Seconds to fall down | Effect of various extracts on sleeping time without Thiopental (min) | Effect of various extracts on Thiopental sleeping time (min) | Score after 24 h |
| I | *A. calamus* | $65^{aaa}$ ±2.0 | $13.4^{aaa}$ ±.1.5 | $138.4^{aaa}$ ±2.4 | 0.00 |
| II | *W. somnifera* | $73^{aaa}$ ±2.0 | $0.7^{aaa}$ ±.1.1 | $123^{cc}$ ±1.2 | 0.00 |
| III | *G. Glabra* | 278±3.0 | 0.00 | 107.8±4.4 | 0.00 |
| IV | A/W/G | $61^{aaa}$ ±3.5 | $15.4^{aaa}$ ±2.1 | $145.8^{aaa}$ ±2.0 | 0.00 |
| V | Marketed formulation | 54.2±2.9 | $23^{cc}$ ±2.6 | $151.4^{aaa}$ ±3.2 | 0.00 |
| VI | Thiopental | - | - | 108.4±3.1 | - |
| VII | Chlorpromazine | $28.4^{aaa}$ ±1.8 | - | - | 3.5 |
| VIII | Control | 300±0.0 | - | - | 0.00 |

Values are expressed as mean ± s.d. of five observations, aaa represents $P < 0.001$ and cc represents $P<0.01$ compared with control. A/W/G stands for *A. calamus*, *W. somnifera* and *G. glabra*.

(righting reflux) was significant, except for *G. glabra*. These results indicate that the tablet formulations with mono ingredient herbal extract were having sufficient neuroleptic effects. The drug induced EPS by seeing the catatonic response were found to be very satisfactory for these formulations with having no catatonia both in mono ingredient and multi ingredient tablet. Therefore, this work suggests that herbal neuroleptic tablet in general and mono ingredient tablet in particular, might have same therapeutic efficacy with no drug induced EPS compared to synthetic chlorpromazine drug.

Safety is a primary consideration for neuroleptic drugs, especially when used in chronic treatment of schizophrenia on a regular basis. An alternative approach of herbal treatment for the same might be beneficial in that aspect. With that view, three neuroleptic plant extracts were used for formulation based on ayurvedic neuroleptic formulations and available literatures. The scientific evaluation was explored to justify the herbal ingredient system of medication in general and single ingredient system in particular. The analytical results obtained from the three plant extracts incorporated in tablet formulation reveal that the active constituents responsible for therapeutic efficacy were very much optimum, compared to the reference standard of marketed products containing same ingredients along with many more plant extracts. When these data were compared over marketed ayurvedic formulation containing several herbal extracts, it was found that these formulations containing three plant extracts and mono herbal preparations were having similar effects as those of marketed formulations. The prepared tablet formulation has not shown any drug induced Parkinsonian syndrome or any other relevant side effects, whereas the synthetic drug, chlorpromazine showed maximum extra pyramidal side effects.

Thus, the present work indicated that mono ingredient system of the herbal tablet formulation is expected to give optimum therapeutic efficacy without any self induced EPS. This may be a better alternative from the marketed polyherbal formulations as well as synthetic drugs used for this purpose. This may be cost effective and affordable neuroleptic formulation during its long maintenance therapy, for psychiatric treatment without any side effects.

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