MATERIALS AND METHODS

लक्षणमात्रोत्तमस्यायत्वो भाव एवं च। सतिमात्रामेत्रेप्रियार्थो वस्त्रिकर्षे न वरसते।।
वैभवमात्रानुसरो ज्ञानमु वस्त्रिकर्ष्यत प्राप्तते। अणुपुत्रमथ चैकार्थे हृ गुणो मनस: स्मृतत।।
Charaka Śāri 1: 18-19.
MATERIALS AND METHODS

The entire study was conducted in two parts, i.e. standardization of the test drug and clinical trial.

(A) TEST DRUG STANDARDIZATION:

Sandalwood oil, often referred to as East Indian Sandalwood oil, is obtained by steam distillation of heartwood of *Santalum album*, L. (Husain, 1994). The essential oil of sandalwood is is extremely viscid, of a light yellow colour and possesses a peculiar, fragrant, characteristic roseate and penetrating odour best appreciated by rubbing a few drops of it on the hand. Oil has a bitterish slightly acrid taste (Dutt, 1995; Sharma, 1999; Nadkarni, 1999). According to standard odour classification, the Sandalwood essential oil has a sweet, woody, balsamic scent, and is considered among the base note – that is, long-staying aroma (Gardiner, 1996).

As far as the standardization of the Sandalwood oil is concerned; in a previous study done by Howes et al. (2004), for setting up of standards associated with the quality of sandalwood oils being traded, specifications of > or = 43% Z-alpha-santalol and > or = 18% Z-beta-santalol for *S. album* oil estimated by GC-MS are suggested. GC-MS was recommended as the most appropriate tool, as it assists with authentication and quality control issues associated with sandalwood oils.

The test drug i.e. essential oil of Chandana (Sandalwood), was procured from the genuine source, through the scientists of Fragrance & Flavour Development Centre (FFDC), Kannauj, U.P., India.

Standardization of the essential oil of Chandana was made to ensure its identity, purity and quality. The authentication of the oil was done through the Holistic Health Care & Research Organization, Pune, India with arrangements of Gas Chromatography made by the Aromatics International Inc., Lolo, Montana, United States (www.AromaticsInternational.com). The sample SAV 103 was standard Sandalwood oil, while the sample SAV 104 was the Sandalwood oil used as a test drug in the present study.
It was observed that the Sandalwood oil used in the study met the standardized criteria of the percentage of the two chief components, i.e. alpha-santalol and beta-santalol those were found 43.30% and 18.40% respectively in the test drug (Sandalwood oil), as compared to the values of the standard sample of Sandalwood oil (alpha-santalol - 45.49% and beta-santalol – 19.30%), as well as previous standard (alpha-santalol - 43% and beta-santalol – 18%) set by Howes et al. (2004).

Aromatics International
www.AromaticsInternational.com

SANTALUM ALBUM
SANDALWOOD (INDIA)
Country of Origin: India

Stock # SAV-103

GAS CHROMATOGRAPHY ANALYSIS (G.C.)

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Stock # SAV-104

GAS CHROMATOGRAPHY ANALYSIS (G.C.)

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Fig. 3: GC-MS Analysis of sandalwood oil (India)

Fig. 3b: Sandalwood oil used in the study
Fig. 4: CHANDANA (Santalum album, Linn.)
Fig. 5: Botanical picture of *Santalum album*, Linn.
(B) CLINICAL TRIAL:

In the survey of literature, in spite of different studies regarding the effect of Sandalwood oil on autonomic nervous system (Block, 2003; Hongratanaworakit et al., 2004; Heuberger, 2006), only one pilot study we found related to the evaluation of the effect of aromatherapy massage with Sandalwood oil in reducing levels of anxiety in palliative care. But due to limited data, the results were not substantial enough to generate coherent statistics; but assumption was made that Sandalwood oil could be effective in reducing anxiety (Kyle, 2006).

As the previous studies have not generated any satisfactory evidences for the anxiolytic effects of Chandana; therefore the present study as a clinical trial was carried out to clinically evaluate the effects of Sandalwood oil, through aroma inhalation, in the patients of generalized anxiety disorder (G.A.D.).

Trial Design:

Controlled, randomized, single-blind, prospective, parallel designed, clinical trial was conducted, as per G.C.P. guidelines.

Sample Size:

The clinical trial was conducted on 100 completed patients.

Ethical clearance:

- Written permission of the Institutional Ethics Committee (IEC) of Tilak Ayurved Mahavidyalaya, Pune was taken (Ref 726 / shark / 72-246 dated 4/10/2006) prior to the initiation of the clinical trial.
- Written permission was obtained from the Principal and Superintendent of Tilak Ayurved Mahavidyalaya, Pune to carry out clinical trials at the Seth Tarachand Ramnath Charitable Ayurvedic Hospital, Pune.
- The Informed Consent Form (Appendix 2) was provided to each individual prior to the initiation of the clinical trial, and every individual
was included in the study only after getting his consent and acceptance for taking the treatment regimen.

**Criteria of Selection (Baseline Screening):**

In the initial (baseline) phase of the clinical trial, the patients were diagnosed through the DSM-IV-TR diagnostic criteria (Hollander and Simeon, 2003) (Appendix 1). The essential feature of the G.A.D., according to DSM-IV, is persistent anxiety lasting at least 6 months. Its symptoms fall within two categories:- 1) apprehensive expectation and worry and 2) physical symptoms those include muscle tension, restlessness, difficulty in concentrating, insomnia, irritability, and fatigue.

Further diagnosis and assessment was done through the Hamilton Rating Scale for Anxiety (HAM-A) that has 14 major components (categories) viz. anxious mood, tension, fears, insomnia, difficulty in concentration, depressed mood, somatic muscular and sensory symptoms; cardiovascular, respiratory, gastrointestinal, genitor-urinary, autonomic and other behavioural symptoms etc. These categories include many sub-parameters (symptoms) to determine their severity.

**Inclusion Criteria:**

1. Patients of same socio-economic status, irrespective of sex and religion. The majority of patients enrolled were from the middle class group.
2. Patients either male or female, between the age group of 25 to 40 years. This age group was included to prevent the inclusion of patients suffering from chronic ailments that occur after the age of 40 years.
3. Informed Consent Form was given to each individual. The patients willingly accepting the treatment were included in the clinical trial.
4. The patients suffering from mild to moderate GAD were included.

**Exclusion Criteria:**

1. The patients having other types of anxiety disorders i.e. anxiety disorder due to general medical condition, panic disorder with or
without agoraphobia, social phobia, specific phobias, separation anxiety disorder, obsessive-compulsive disorder (OCD), substance-induced anxiety disorder, post-traumatic stress disorder (PTSD) and acute stress disorder were not included in the study.

2. The patients having secondary GAD due to neurological disorders, systemic conditions, endocrine disturbances, inflammatory disorders, deficiency states and miscellaneous conditions were excluded from the study.

3. The patients suffering from severe GAD were excluded from the study.

4. The patients having tuberculosis, diabetes, hypertension, renal disorders, cardiac disorders and other psychiatric problems and life threatening diseases were excluded from the study.

5. The patients not willingly accepting the treatment were excluded from the clinical trial.

**Grouping:**

Total 110 individuals were recruited for the study. But the study was done finally on 100 completed patients. They were divided by random sampling, according to lottery method, into following groups, comprising of 50 individuals in each group.

**Test Group:** Sandalwood oil + Placebo (starch) capsules

**Control Group:** Placebo (starch) capsules

The placebo was given in both the groups to nullify the bias of the effect of counseling during the treatment phase.

**Dosage and Route of administration:**

- Two drops (0.1 ml.) Sandalwood oil with one ml. of distilled water, through nebulizer for 5 minutes, twice a day.
- Placebo (starch) capsule of 500 mg. orally, B.D.

**Time of Administration:**

Sandalwood oil by inhalation and starch capsules orally, after breakfast (9 A.M.) and evening (5 P.M.).
Duration of administration of the drugs:

Total duration of study was two months. However, the follow-up was done for a period of another one month (at every 15 days) to assess the relapse cases.

Drop-outs:
1. Patients following the treatment very irregularly
2. Patients absent in further follow-ups

Follow ups:

Follow ups were carried out on each fifteenth days for two months, and after the completion further at one month.

ASSESSMENT CRITERIA:

The assessments were made on each fifteen days, on the basis of the following parameters.

1. Case Report Form (CRF):

The patients were subjected to detailed clinical history and physical examination. The Daśavidha Parikṣa was performed, in which the parameters observed were viz. Prakṛti, Vikṛti, Sāra, Saṁthananā, Pramāṇa, Satmya, Satvā, Ahāra Śakti, Vyayama Śakti and Vaya (Appendix 3, 4, 5 & 6). The Prakṛti Parikṣan (investigation for constitution) was done according to the proforma (Appendix 4) described by Srikantamurthy (1996), that has been developed by him on the basis of classical Ayurvedic literature (Cha. Vim. 8/96-99; Su. Sha. 4/; Ash. San. Sha. 8/; Ash. Hr. Sha. 3).

2. Psychometric Analysis of Generalized Anxiety Disorder (GAD):

The Hamilton Rating Scale for Anxiety (HAM-A) (Sajatovic and Ramirez, 2003) was used for the assessment of signs and symptoms of the G.A.D. The standardized Hindi version (Appendix 7) was used as a questionnaire to be given to the patients. The quantification / rating of the severity of signs and symptoms was done as: - very severe (4), severe (3), moderate (2), mild (1) and none (0).
Criteria for assessing the anxiety was followed as - mild anxiety (minimum 18 up to 24); moderate anxiety (minimum 25 up to 29); and severe anxiety (minimum 30 and above).

**Statistical Analysis:**

The analysis of all the parameters was done statistically by the One-way Analysis of Variance (ANOVA) – Dunnett Multiple Comparisons Test using “Graph Pad InStat” of the Graph Pad Software Inc., San Diego, U.S.A. All the values were expressed as mean ± S.E.M. Probability level of less than 5% was considered as statistically significant.

**Adverse Drug Reactions (ADR):**

The ADR of the test drug on the patients taking treatment were checked if any! The individuals having mild reactions, like mucosal irritation, sneezing, rhinorrhea etc. were continued for the treatment.

**Criteria of Withdrawal:**

1. The patients having moderate to severe adverse effects of the Test Drug were planned to be withdrawn from the treatment, and they were further followed for some period to see either the negative effects were continued or not.
2. The patients unwilling to continue the treatment were discontinued and withdrawn from the study.